05-1 JC17 Rec'd PCT/PTO 10 MAY 2001

FORM PTO-1390 (Modified) (REV 10-95) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

OIPE MAY 10 2001 PATENT & TRADEMARK OFFICE

ATTORNEY'S DOCKET NUMBER	U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR)
BB-1270	09/831683

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/US99/26478	9 NOVEMBER 1999 (09.09.99)	10 NOVEMBER 1998 (10.11.98)

TITLE OF INVENTION
PLANT AMINOACYL-tRNA SYNTHETASES

APPLICANT(S) FOR DO/EO/US
FAMODU, Omolayo O. et al.

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information

1. ☑ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☑ This is an express request to being national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b)) and PCT Articles 22 and 39(1).
4. ☑ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ☑ A copy of the International Application was filed (35 U.S.C. 371 (c) (2))
   a. ☒ is transmitted herewith (required only if not transmitted by the International Bureau.
   b. ☐ has been transmitted by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US)
6. ☐ A translation of the International Application into English (35 U.S.C. 371 (c) (2)).
7. ☑ A copy of the International Search Report (PCT/ISA/210).
8. ☑ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c) (3))
   a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ have been transmitted by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☒ have not been made and will not be made.
9. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371 (c)(3)).
10. ☑ An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)).
11. ☑ A copy of the International Preliminary Examination Report (PCT/IPEA/409)
12. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).

Items 13 to 18 below concern document(s) or information included :

13. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
14. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
15. ☐ A FIRST preliminary amendment.
    A SECOND or SUBSEQUENT preliminary amendment.
16. ☐ A substitute specification.
17. ☑ A change of power of attorney and/or address letter.
18. ☑ Certificate of Mailing by Express Mail.
19. ☐ Other items or information:

17. General Power of Attorney
18. Express Mailing Label No.: EI182981146US

Mailed 5/10/01

JC08 Rec'd PCT/PTO 10 MAY 2001

APPLICATION NO. (IF KNOWN, SEE 37 CFR)	INTERNATIONAL APPLICATION NO.	ATTORNEY'S DOCKET NUMBER
09/831683	PCT/US99/26478	BB-1270

20. The following fees are submitted

CALCULATIONS PTO USE ONLY

BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) – (5)) :

☑ Search Report has been prepared by the EPO or JPO	$860.00	
☐ International preliminary examination fee paid to USPTO (37 CFR 1.482)	$690.00	
☐ No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2))	$760.00	
☐ Neither international preliminary examination fee paid to USPTO (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO	$1000.00	
☐ International preliminary examination fee paid to USPTO (37 CFR 1.482) And all claims satisfied provisions of PCT Article 33(2)-(4)	$ 100.00	
ENTER APPROPRIATE BASIC FEE AMOUNT =		**$860.00**
Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (e)).		**$0.00**

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE	
Total Claims	**69** - 20 =	**49** x	$18.00	**$882.00**
Independent Claims	**13** - 3 =	**49** x	$80.00	**$800.00**
Multiple Dependent Claims **(check if applicable)**			☒	**$270.00**

TOTAL OF ABOVE CALCULATIONS =	**$1,952.00**
Reduction of ½ for filing by small entity, if applicable. Verified Small Entity Statement must also be filed (Note 37 CFR 1.9, 1.27, 1.28) **(check if applicable).** ☐	**$0.00**
SUBTOTAL =	**$1,952.00**
Processing Fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (f)).	**$0.00**
TOTAL NATIONAL FEE =	**$2,812.00**
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) **(check if applicable).** ☐	**$0.00**
TOTAL FEES ENCLOSED =	**$2,812.00**
	Amount to be : refunded **$**
	Charged **$**

☐ A check in the amount of _____ to cover the above fees enclosed.

☑ Please charge my Deposit Account No. **04-1928** in the amount of **$2,812.00** to cover the above fees.

☑ The Commissioner is hereby authorized to charge any fees which may be required, or credit any overpayment to Deposit Account No. **04-1928** a duplicate copy of this sheet is enclosed.

NOTE : Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (CFR 1.37(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

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SIGNATURE Andrew L. Schaeffer
for **RIZZO, THOMAS M.**
NAME
41,272
REGISTRATION NUMBER
10 May 2001
DATE

Rec'd PCT/PTO 15 AUG 2001

Rev. 10/93

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the Application of:

O. FAMODU ET AL. CASE NO.: BB1270

APPLICATION NO.: 09/831,683 GROUP ART UNIT: UNKNOWN

INTERNATIONAL FILING DATE: 09 NOV 99 EXAMINER: UNKNOWN

FOR: PLANT AMINOACYL-tRNA SYNTHETASES

PRELIMINARY AMENDMENT AND RESPONSE TO NOTIFICATION OF MISSING REQUIREMENTS UNDER 35 U.S.C. 371

Assistant Commissioner for Patents
Box PCT
Washington, D.C. 20231

Sir:

In response to a Notification of Missing Requirements under 35 U.S.C. 371 dated 13 June 2001, please amend the application as follows.

IN THE SPECIFICATION

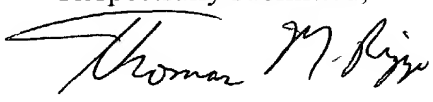
Please replace the originally filed Sequence Listing with the attached substitute Sequence Listing.

REMARKS

Please charge any necessary fee to Deposit Account 04-1928 E. I. du Pont de Nemours and Company).

In view of the foregoing, allowance of the above-referenced application is respectfully requested.

Respectfully submitted,



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Dated: August 8, 2001

09831683 051000
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09831683

TITLE

PLANT AMINOACYL-tRNA SYNTHETASES

This application claims the benefit of U.S. Provisional Application No. 60/107,789, filed November 10, 1998.

FIELD OF THE INVENTION

This invention is in the field of plant molecular biology. More specifically, this invention pertains to nucleic acid fragments encoding aminoacyl-tRNA synthetases in plants and seeds.

BACKGROUND OF THE INVENTION

Aminoacyl-tRNA Synthetases (AARS) are enzymes that charge (acylate) tRNAs with amino acids. These charged aminoacyl tRNAs then participate in mRNA translation and protein synthesis. The AARS show high specificity for charging a specific tRNA with the appropriate amino acid, for example valyl-tRNA with valine by valyl-tRNA synthetase or tryptophanyl-tRNA with tryptophan by tryptophanyl-tRNA synthetase. In general, per organism there are at least one AARS for each of the twenty amino acids. There are exceptions however. AARS are ancient enzymes, having functioned in translation since early life evolution. Some have speculated that the earliest aminoacyl-tRNA synthetases were mRNAs, not proteins, with the proteinaceous AARS described here emerging later (Neidhardt et al., (1975) *Annu. Rev. Microbiol. 29*:215-250). AARS are structurally diverse, although AARSs for some amino acids are more closely related than for others. AARSs are generally divided into two classes, class I and class II based on structural similarity and amino acid preferences (Eriani et al., (1990) *Nature 347*:203-206).

Plants like all other cellular organisms have aminoacyl-tRNA synthetases. However, a full description of the plant 'complement' of aminoacyl-tRNA synthetases has not yet been described. Full-length cDNA, genomic clones, and EST sequences for a variety of plant aminoacyl-tRNA synthetases are known. However, several anticipated aminoacyl-tRNA synthetases have not been discovered.

Because of the central role of protein synthesis in life, any agent that inhibits or disrupts this activity is likely to be toxic. Aminoacyl-tRNA synthetases play a critical role in protein translation by linking genetic nucleic acid information to protein synthesis. Aminoacyl-tRNA synthetases perform this role by "reading" the identity of the different tRNAs and acylating them with the correct cognate amino acid. A large volume of research over several decades has been focused on identifying inhibitors of this process. Inhibitors of aminoacyl-tRNA synthetases have been found to be cytotoxic due to their inhibition of protein synthesis. As such they therefore could be used as herbicides or in aminoacyl-tRNA synthetase selectable marker systems (Lloyd et al., (1995) Nucleic Acid Research 23(15):2882-2892). The genes disclosed herein can serve as the basis for testing

whether the encoded aminoacyl-tRNA synthetases are sensitive to known inhibitors or other chemicals.

Biochemical processes are often compartmentalized in regions of cells, such as mitochondria, plastids, and lysosomes. These organelles are key sites for many biochemical pathways. Bioengineering of these processes may require targeting protein products to specific organells. One method to accomplish this involves the addition of an N-terminal prosequence (transit peptide) that directs protein entry into a specific organelle(s). Upon or shortly after transport into the organelle the transit peptide is usually proteolytically removed, and the mature protein is then able to function.

A few plant transit peptides have been shown empirically to be capable of directing fused proteins into specific organelles. However this ability appears to depend upon the structure of the protein being imported and to date it is impossible to predict whether a protein will be imported into an organelle with a given transit peptide. As such, it is advantageous to have a diversity of potential transit peptides from which the most efficient candidate can be chosen to target a protein of interest to an organelle. A number of plant transit peptides are known which direct mature proteins to mitochondria or chloroplast organells. These transit peptides are diverse in structure (length and amino acid sequence) and there is no strong consensus sequence identifying them. In addition, there is no obvious clear relationship between chloroplast targeting and mitochondrial targeting transit sequences. This invention describes a number of chloroplast-targeting and mitochondria-targeting transit peptides for (maize) aminoacyl-tRNA synthetases. These sequences will find utility in directing both aminoacyl-tRNA synthetase and other proteins into these organelles.

Accordingly, the availability of nucleic acid sequences encoding all or a portion of these enzymes would facilitate studies to better understand protein synthesis in plants, provide genetic tools for the manipulation of gene expression, protein targeting to specific organells and provide possible targets for herbicides.

SUMMARY OF THE INVENTION

The present invention relates to isolated polynucleotides comprising a nucleotide sequence encoding a polypeptide of at least 240 amino acids that has at least 80% identity based on the Clustal method of alignment when compared to a polypeptide selected from the group consisting of a corn arginyl-tRNA synthetase polypeptide of SEQ ID NO:2, a rice arginyl-tRNA synthetase polypeptide of SEQ ID NO:4, a soybean arginyl-tRNA synthetase polypeptide of SEQ ID NO:6, a wheat arginyl-tRNA synthetase polypeptide of SEQ ID NO:8. The present invention also relates to an isolated polynucleotide comprising the complement of the nucleotide sequences described above.

The present invention also relates to isolated polynucleotides comprising a nucleotide sequence encoding a polypeptide of at least 205 amino acids that has at least 90% identity

based on the Clustal method of alignment when compared to a polypeptide selected from the group consisting of a corn glutamyl-tRNA synthetase polypeptide of SEQ ID NO:10, a rice glutamyl-tRNA synthetase polypeptide of SEQ ID NO:12, a soybean glutamyl-tRNA synthetase polypeptide of SEQ ID NO:14. The present invention also relates to an isolated polynucleotide comprising the complement of the nucleotide sequences described above.

The present invention also relates to isolated polynucleotides comprising a nucleotide sequence encoding a polypeptide of at least 79 amino acids that has at least 80% identity based on the Clustal method of alignment when compared to a polypeptide selected from the group consisting of a wheat glutamyl-tRNA synthetase polypeptide of SEQ ID NO:16. The present invention also relates to an isolated polynucleotide comprising the complement of the nucleotide sequences described above.

The present invention further relates to isolated polynucleotides comprising a nucleotide sequence encoding a polypeptide of at least 243 amino acids that has at least 90% identity based on the Clustal method of alignment when compared to a polypeptide selected from the group consisting of a corn histidyl-tRNA synthetase polypeptide of SEQ ID NO:18, a soybean histidyl-tRNA synthetase polypeptide of SEQ ID NO:20, a wheat histidyl-tRNA synthetase polypeptide of SEQ ID NO:22. The present invention also relates to an isolated polynucleotide comprising the complement of the nucleotide sequences described above.

The present invention relates to isolated polynucleotides comprising a nucleotide sequence encoding a polypeptide of at least 30 amino acids that has at least 60% identity based on the Clustal method of alignment when compared to a polypeptide selected from the group consisting of SEQ ID NOs:24, 26, 28, 30, 32, 34, 36 and 38.

It is preferred that the isolated polynucleotides of the claimed invention consists of a nucleic acid sequence selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35 and 37 that codes for the polypeptide selected from the group consisting of SEQ ID NOs:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36 and 38. The present invention also relates to an isolated polynucleotide comprising a nucleotide sequences of at least one of 60 (preferably at least one of 40, most preferably at least one of 30) contiguous nucleotides derived from a nucleotide sequence selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37 and the complement of such nucleotide sequences.

The present invention relates to a chimeric gene comprising an isolated polynucleotide of the present invention operably linked to suitable regulatory sequences.

The present invention also relates to the identification of transit peptides associated with aminoacyl-tRNA synthetases of the instant invention and the use of those transit peptides to target aminoacyl-tRNA synthetases and other operably linked proteins to specific organelles within plant cells. Transit peptide amino acid sequences are located just upstream

of the mature aminoacyl-tRNA synthetase polypeptide sequences disclosed in the instant invention.

The present invention relates to an isolated host cell comprising a chimeric gene of the present invention or an isolated polynucleotide of the present invention. The host cell may be eukaryotic, such as a yeast or a plant cell, or prokaryotic, such as a bacterial cell. The present invention also relates to a virus, preferably a baculovirus, comprising an isolated polynucleotide of the present invention or a chimeric gene of the present invention.

The present invention relates to a process for producing an isolated host cell comprising a chimeric gene of the present invention or an isolated polynucleotide of the present invention, the process comprising either transforming or transfecting an isolated compatible host cell with a chimeric gene or isolated polynucleotide of the present invention.

The present invention relates to an arginyl-tRNA synthetase polypeptide of at least 240 amino acids comprising at least 80% homology based on the Clustal method of alignment compared to a polypeptide selected from the group consisting of SEQ ID NOs:2, 4, 6, and 8.

The present invention relates to a glutamyl-tRNA synthetase polypeptide of at least 205 amino acids comprising at least 90% homology based on the Clustal method of alignment compared to a polypeptide selected from the group consisting of SEQ ID NOs:10, 12, 14 and 16.

The present invention relates to a glutamyl-tRNA synthetase polypeptide of at least 79 amino acids comprising at least 80% homology based on the Clustal method of alignment compared to a polypeptide of SEQ ID NO:16.

The present invention relates to a histidyl-tRNA synthetase polypeptide of at least 243 amino acids comprising at least 90% homology based on the Clustal method of alignment compared to a polypeptide selected from the group consisting of SEQ ID NOs:18, 20 and 22.

The present invention relates to a method of selecting an isolated polynucleotide that affects the level of expression of an arginyl-tRNA synthetase, glutamyl-tRNA synthetase or histidyl-tRNA synthetase polypeptide in a host cell, preferably a plant cell, the method comprising the steps of:

constructing an isolated polynucleotide of the present invention or an isolated chimeric gene of the present invention;

introducing the isolated polynucleotide or the isolated chimeric gene into a host cell;

measuring the level an arginyl-tRNA synthetase, glutamyl-tRNA synthetase or histidyl-tRNA synthetase polypeptide in the host cell containing the isolated polynucleotide; and

comparing the level of an arginyl-tRNA synthetase, glutamyl-tRNA synthetase or histidyl-tRNA synthetase polypeptide in the host cell containing the isolated polynucleotide with the level of an arginyl-tRNA synthetase, glutamyl-tRNA synthetase or histidyl-tRNA synthetase polypeptide in a host cell that does not contain the isolated polynucleotide.

The present invention relates to a method of obtaining a nucleic acid fragment encoding a substantial portion of an arginyl-tRNA synthetase, glutamyl-tRNA synthetase or histidyl-tRNA synthetase polypeptide gene, preferably a plant arginyl-tRNA synthetase, glutamyl-tRNA synthetase or histidyl-tRNA synthetase polypeptide gene, comprising the steps of: synthesizing an oligonucleotide primer comprising a nucleotide sequence of at least one of 60 (preferably at least one of 40, most preferably at least 30) contiguous nucleotides derived from a nucleotide sequence selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37 and the complement of such nucleotide sequences; and amplifying a nucleic acid fragment (preferably a cDNA inserted in a cloning vector) using the oligonucleotide primer. The amplified nucleic acid fragment preferably will encode a portion of an arginyl-tRNA synthetase, glutamyl-tRNA synthetase or histidyl-tRNA synthetase amino acid sequence.

The present invention also relates to a method of obtaining a nucleic acid fragment encoding all or a substantial portion of the amino acid sequence encoding an arginyl-tRNA synthetase, glutamyl-tRNA synthetase or histidyl-tRNA synthetase polypeptide comprising the steps of: probing a cDNA or genomic library with an isolated polynucleotide of the present invention; identifying a DNA clone that hybridizes with an isolated polynucleotide of the present invention; isolating the identified DNA clone; and sequencing the cDNA or genomic fragment that comprises the isolated DNA clone.

A further embodiment of the instant invention is a method for evaluating at least one compound for its ability to inhibit the activity of an arginyl-tRNA synthetase, glutamyl-tRNA synthetase or histidyl-tRNA synthetase, the method comprising the steps of:
(a) transforming a host cell with a chimeric gene comprising a nucleic acid fragment encoding an arginyl-tRNA synthetase, glutamyl-tRNA synthetase or histidyl-tRNA synthetase, operably linked to suitable regulatory sequences; (b) growing the transformed host cell under conditions that are suitable for expression of the chimeric gene wherein expression of the chimeric gene results in production of arginyl-tRNA synthetase, glutamyl-tRNA synthetase or histidyl-tRNA synthetase in the transformed host cell; (c) optionally purifying the arginyl-tRNA synthetase, glutamyl-tRNA synthetase or histidyl-tRNA synthetase expressed by the transformed host cell; (d) treating the arginyl-tRNA synthetase, glutamyl-tRNA synthetase or histidyl-tRNA synthetase with a compound to be tested; and (e) comparing the activity of the arginyl-tRNA synthetase, glutamyl-tRNA synthetase or histidyl-tRNA synthetase that has been treated with a test compound to the activity of an

untreated arginyl-tRNA synthetase, glutamyl-tRNA synthetase or histidyl-tRNA synthetase, thereby selecting compounds with potential for inhibitory activity.

The present invention relates to a composition comprising an isolated polynucleotide of the present invention.

The present invention relates to a composition comprising a polypeptide of the present invention.

The present invention relates to an isolated polynucleotide comprising the nucleotide sequence comprising at least one of 30 contiguous nucleotides of nucleic acid sequences selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37 and the complement of such sequences.

The present invention relates to an expression cassette comprising an isolated polynucleotide of the present invention operably linked to a promoter.

The present invention relates to a method for positive selection of a transformed cell comprising:

(a) transforming a plant cell with an expression cassette of the present invention;

(b) growing the transformed plant cell under conditions allowing expression of the polynucleotide in an amount sufficient to complement an amino-acyl t-RNA synthesis auxotroph in a plant cell to provide a positive selection means.

The present invention relates to a method for positive selection of a transformed cell comprising:

(a) transforming a plant cell with a chimeric gene of the present invention; and

(b) growing the transformed plant cell, wherein the plant cell is a monocot or a dicot and includes corn, rice, soybean or wheat under conditions allowing expression of the polynucleotide in an amount sufficient to complement an amino-acyl t-RNA synthesis.

BRIEF DESCRIPTION OF THE SEQUENCE DESCRIPTIONS

The invention can be more fully understood from the following detailed description and the accompanying Sequence Listing which form a part of this application.

Table 1 lists the polypeptides that are described herein, the designation of the cDNA clones that comprise the nucleic acid fragments encoding polypeptides representing all or a substantial portion of these polypeptides, and the corresponding identifier (SEQ ID NO:) as used in the attached Sequence Listing. Table 1 also identifies the cDNA clones as individual ESTs ("EST"), the sequences of the entire cDNA inserts comprising the indicated cDNA clones ("FIS"), contigs assembled from two or more ESTs ("Contig"), contigs assembled from an FIS and one or more ESTs ("Contig*"), or sequences encoding the entire protein derived from an FIS, a contig, or an FIS and PCR ("CGS"). Nucleotide sequences, SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, 19 and 21 and amino acid sequences SEQ ID NOs:2, 4, 6, 8, 10, 12, 14, 16, 18, 20 and 22 were determined by

further sequence analysis of cDNA clones encoding the amino acid sequences set forth in SEQ ID NOs:24, 26, 28, 30, 32, 34, 36 and 38. Nucleotide SEQ ID NOs:23, 25, 27, 29, 31, 33, 35 and 37 and amino acid SEQ ID NOs:24, 26, 28, 30, 32, 34, 36 and 38 were presented in a U.S. Provisional Application No. 60/107,789, filed November 10, 1998.

The sequence descriptions and Sequence Listing attached hereto comply with the rules governing nucleotide and/or amino acid sequence disclosures in patent applications as set forth in 37 C.F.R. §1.821-1.825.

TABLE 1

Aminoacyl-tRNA Synthetases

Protein	Clone Designation	SEQ ID NO: (Nucleotide)	(Amino Acid)
Arginyl-tRNA Synthetase	cpc1c.pk001.d11 (FIS)	1	2
Arginyl-tRNA Synthetase	rl0n.pk086.p16 (FIS)	3	4
Arginyl-tRNA Synthetase	ssm.pk0026.b10 (FIS)	5	6
Arginyl-tRNA Synthetase	wlk1.pk0007.f5 (FIS)	7	8
Glutamyl-tRNA Synthetase	p0010.cbpcp10r (CGS)	9	10
Glutamyl-tRNA Synthetase	rlr2.pk0032.f2 (CGS)	11	12
Glutamyl-tRNA Synthetase	Contig Composed of: sdc5c.pk0002.e11 sgs1c.pk001.k12 sgs1c.pk004.e20	13	14
Glutamyl-tRNA Synthetase	wlm96.pk055.g5 (EST)	15	16
Histidyl-tRNA Synthetase	p0102.cerbb73r (CGS)	17	18
Histidyl-tRNA Synthetase	Contig composed of: sdp4c.pk007.c7 ssm.pk0012.d9	19	20
Histidyl-tRNA Synthetase	wr1.pk0079.d1 (FIS)	21	22
Arginyl-tRNA Synthetase	cpc1c.pk001.d11 (EST)	23	24
Arginyl-tRNA Synthetase	rl0n.pk086.p16 (EST)	25	26
Arginyl-tRNA Synthetase	ssm.pk0026.b10 (EST)	27	28
Arginyl-tRNA Synthetase	wlk1.pk0007.f5 (EST)	29	30
Glutamyl-tRNA Synthetase	rlr2.pk0032.f2 (EST)	31	32
Glutamyl-tRNA Synthetase	sgs1c.pk004.e20 (EST)	33	34
Histidyl-tRNA Synthetase	ssm.pk0012.d9 (EST)	35	36
Histidyl-tRNA Synthetase	wr1.pk0079.d1 (EST)	37	38

The Sequence Listing contains the one letter code for nucleotide sequence characters and the three letter codes for amino acids as defined in conformity with the IUPAC-IUBMB standards described in *Nucleic Acids Res. 13*:3021-3030 (1985) and in the *Biochemical J. 219 (No. 2)*:345-373 (1984) which are herein incorporated by reference. The symbols and format used for nucleotide and amino acid sequence data comply with the rules set forth in 37 C.F.R. §1.822.

DETAILED DESCRIPTION OF THE INVENTION

In the context of this disclosure, a number of terms shall be utilized. As used herein, a "polynucleotide" is a nucleotide sequence such as a nucleic acid fragment. A polynucleotide may be a polymer of RNA or DNA that is single- or double-stranded, that optionally contains synthetic, non-natural or altered nucleotide bases. A polynucleotide in the form of a polymer of DNA may be comprised of one or more segments of cDNA, genomic DNA, or synthetic DNA. An isolated polynucleotide of the present invention may include at least one of 60 contiguous nucleotides, preferably at least one of 40 contiguous nucleotides, most preferably one of at least 30 contiguous nucleotides, of the nucleic acid sequence of the SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37 and the complement of such sequences.

As used herein, "contig" refers to a nucleotide sequence that is assembled from two or more constituent nucleotide sequences that share common or overlapping regions of sequence homology. For example, the nucleotide sequences of two or more nucleic acid fragments can be compared and aligned in order to identify common or overlapping sequences. Where common or overlapping sequences exist between two or more nucleic acid fragments, the sequences (and thus their corresponding nucleic acid fragments) can be assembled into a single contiguous nucleotide sequence.

As used herein, "substantially similar" refers to nucleic acid fragments wherein changes in one or more nucleotide bases results in substitution of one or more amino acids, but do not affect the functional properties of the polypeptide encoded by the nucleotide sequence. "Substantially similar" also refers to nucleic acid fragments wherein changes in one or more nucleotide bases does not affect the ability of the nucleic acid fragment to mediate alteration of gene expression by gene silencing through for example antisense or co-suppression technology. "Substantially similar" also refers to modifications of the nucleic acid fragments of the instant invention such as deletion or insertion of one or more nucleotides that do not substantially affect the functional properties of the resulting transcript vis-à-vis the ability to mediate gene silencing or alteration of the functional properties of the resulting protein molecule. It is therefore understood that the invention encompasses more than the specific exemplary nucleotide or amino acid sequences and includes functional equivalents thereof.

Substantially similar nucleic acid fragments may be selected by screening nucleic acid fragments representing subfragments or modifications of the nucleic acid fragments of the instant invention, wherein one or more nucleotides are substituted, deleted and/or inserted, for their ability to affect the level of the polypeptide encoded by the unmodified nucleic acid fragment in a plant or plant cell. For example, a substantially similar nucleic acid fragment representing at least one of 30 contiguous nucleotides derived from the instant nucleic acid fragment can be constructed and introduced into a plant or plant cell. The level of the polypeptide encoded by the unmodified nucleic acid fragment present in a plant or plant cell exposed to the substantially similar nucleic fragment can then be compared to the level of the polypeptide in a plant or plant cell that is not exposed to the substantially similar nucleic acid fragment.

For example, it is well known in the art that antisense suppression and co-suppression of gene expression may be accomplished using nucleic acid fragments representing less than the entire coding region of a gene, and by nucleic acid fragments that do not share 100% sequence identity with the gene to be suppressed. Moreover, alterations in a nucleic acid fragment which result in the production of a chemically equivalent amino acid at a given site, but do not effect the functional properties of the encoded polypeptide, are well known in the art. Thus, a codon for the amino acid alanine, a hydrophobic amino acid, may be substituted by a codon encoding another less hydrophobic residue, such as glycine, or a more hydrophobic residue, such as valine, leucine, or isoleucine. Similarly, changes which result in substitution of one negatively charged residue for another, such as aspartic acid for glutamic acid, or one positively charged residue for another, such as lysine for arginine, can also be expected to produce a functionally equivalent product. Nucleotide changes which result in alteration of the N-terminal and C-terminal portions of the polypeptide molecule would also not be expected to alter the activity of the polypeptide. Each of the proposed modifications is well within the routine skill in the art, as is determination of retention of biological activity of the encoded products. Consequently, an isolated polynucleotide comprising a nucleotide sequence of at least one of 60 (preferably at least one of 40, most preferably at least one of 30) contiguous nucleotides derived from a nucleotide sequence selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37 and the complement of such nucleotide sequences may be used in methods of selecting an isolated polynucleotide that affects the expression of a polypeptide in a plant cell. A method of selecting an isolated polynucleotide that affects the level of expression of a polypeptide (such as aminoacyl-tRNA synthetase) in a host cell (eukaryotic, such as plant or yeast, prokaryotic such as bacterial, or viral) may comprise the steps of: constructing an isolated polynucleotide of the present invention or an isolated chimeric gene of the present invention; introducing the isolated polynucleotide or the isolated chimeric gene into a host cell; measuring the level a polypeptide in the host cell containing the

isolated polynucleotide; and comparing the level of a polypeptide in the host cell containing the isolated polynucleotide with the level of a polypeptide in a host cell that does not contain the isolated polynucleotide.

Moreover, substantially similar nucleic acid fragments may also be characterized by their ability to hybridize. Estimates of such homology are provided by either DNA-DNA or DNA-RNA hybridization under conditions of stringency as is well understood by those skilled in the art (Hames and Higgins, Eds. (1985) Nucleic Acid Hybridisation, IRL Press, Oxford, U.K.). Stringency conditions can be adjusted to screen for moderately similar fragments, such as homologous sequences from distantly related organisms, to highly similar fragments, such as genes that duplicate functional enzymes from closely related organisms. Post-hybridization washes determine stringency conditions. One set of preferred conditions uses a series of washes starting with 6X SSC, 0.5% SDS at room temperature for 15 min, then repeated with 2X SSC, 0.5% SDS at 45°C for 30 min, and then repeated twice with 0.2X SSC, 0.5% SDS at 50°C for 30 min. A more preferred set of stringent conditions uses higher temperatures in which the washes are identical to those above except for the temperature of the final two 30 min washes in 0.2X SSC, 0.5% SDS was increased to 60°C. Another preferred set of highly stringent conditions uses two final washes in 0.1X SSC, 0.1% SDS at 65°C.

Substantially similar nucleic acid fragments of the instant invention may also be characterized by the percent identity of the amino acid sequences that they encode to the amino acid sequences disclosed herein, as determined by algorithms commonly employed by those skilled in this art. Suitable nucleic acid fragments (isolated polynucleotides of the present invention) encode polypeptides that are at least 70% identical, preferably at least 80% identical to the amino acid sequences reported herein. Preferred nucleic acid fragments encode amino acid sequences that are at least 85% identical to the amino acid sequences reported herein. More preferred nucleic acid fragments encode amino acid sequences that are at least 90% identical to the amino acid sequences reported herein. Most preferred are nucleic acid fragments that encode amino acid sequences that are at least 95% identical to the amino acid sequences reported herein. Suitable nucleic acid fragments not only have the above homologies but typically encode a polypeptide having at least 50 amino acids, preferably at least 100 amino acids, more preferably at least 150 amino acids, still more preferably at least 200 amino acids, and most preferably at least 250 amino acids. Sequence alignments and percent identity calculations were performed using the Megalign program of the LASERGENE bioinformatics computing suite (DNASTAR Inc., Madison, WI). Multiple alignment of the sequences was performed using the Clustal method of alignment (Higgins and Sharp (1989) *CABIOS. 5*:151-153) with the default parameters (GAP PENALTY=10, GAP LENGTH PENALTY=10). Default parameters for pairwise

alignments using the Clustal method were KTUPLE 1, GAP PENALTY=3, WINDOW=5 and DIAGONALS SAVED=5.

A "substantial portion" of an amino acid or nucleotide sequence comprises an amino acid or a nucleotide sequence that is sufficient to afford putative identification of the protein or gene that the amino acid or nucleotide sequence comprises. Amino acid and nucleotide sequences can be evaluated either manually by one skilled in the art, or by using computer-based sequence comparison and identification tools that employ algorithms such as BLAST (Basic Local Alignment Search Tool; Altschul et al. (1993) *J. Mol. Biol. 215*:403-410; see also www.ncbi.nlm.nih.gov/BLAST/). In general, a sequence of ten or more contiguous amino acids or thirty or more contiguous nucleotides is necessary in order to putatively identify a polypeptide or nucleic acid sequence as homologous to a known protein or gene. Moreover, with respect to nucleotide sequences, gene-specific oligonucleotide probes comprising 30 or more contiguous nucleotides may be used in sequence-dependent methods of gene identification (e.g., Southern hybridization) and isolation (e.g., *in situ* hybridization of bacterial colonies or bacteriophage plaques). In addition, short oligonucleotides of 12 or more nucleotides may be used as amplification primers in PCR in order to obtain a particular nucleic acid fragment comprising the primers. Accordingly, a "substantial portion" of a nucleotide sequence comprises a nucleotide sequence that will afford specific identification and/or isolation of a nucleic acid fragment comprising the sequence. The instant specification teaches amino acid and nucleotide sequences encoding polypeptides that comprise one or more particular plant proteins. The skilled artisan, having the benefit of the sequences as reported herein, may now use all or a substantial portion of the disclosed sequences for purposes known to those skilled in this art. Accordingly, the instant invention comprises the complete sequences as reported in the accompanying Sequence Listing, as well as substantial portions of those sequences as defined above.

"Codon degeneracy" refers to divergence in the genetic code permitting variation of the nucleotide sequence without effecting the amino acid sequence of an encoded polypeptide. Accordingly, the instant invention relates to any nucleic acid fragment comprising a nucleotide sequence that encodes all or a substantial portion of the amino acid sequences set forth herein. The skilled artisan is well aware of the "codon-bias" exhibited by a specific host cell in usage of nucleotide codons to specify a given amino acid. Therefore, when synthesizing a nucleic acid fragment for improved expression in a host cell, it is desirable to design the nucleic acid fragment such that its frequency of codon usage approaches the frequency of preferred codon usage of the host cell.

"Synthetic nucleic acid fragments" can be assembled from oligonucleotide building blocks that are chemically synthesized using procedures known to those skilled in the art. These building blocks are ligated and annealed to form larger nucleic acid fragments which may then be enzymatically assembled to construct the entire desired nucleic acid fragment.

"Chemically synthesized", as related to nucleic acid fragment, means that the component nucleotides were assembled *in vitro*. Manual chemical synthesis of nucleic acid fragments may be accomplished using well established procedures, or automated chemical synthesis can be performed using one of a number of commercially available machines. Accordingly, the nucleic acid fragments can be tailored for optimal gene expression based on optimization of nucleotide sequence to reflect the codon bias of the host cell. The skilled artisan appreciates the likelihood of successful gene expression if codon usage is biased towards those codons favored by the host. Determination of preferred codons can be based on a survey of genes derived from the host cell where sequence information is available.

"Gene" refers to a nucleic acid fragment that expresses a specific protein, including regulatory sequences preceding (5' non-coding sequences) and following (3' non-coding sequences) the coding sequence. "Native gene" refers to a gene as found in nature with its own regulatory sequences. "Chimeric gene" refers any gene that is not a native gene, comprising regulatory and coding sequences that are not found together in nature. Accordingly, a chimeric gene may comprise regulatory sequences and coding sequences that are derived from different sources, or regulatory sequences and coding sequences derived from the same source, but arranged in a manner different than that found in nature. "Endogenous gene" refers to a native gene in its natural location in the genome of an organism. A "foreign" gene refers to a gene not normally found in the host organism, but that is introduced into the host organism by gene transfer. Foreign genes can comprise native genes inserted into a non-native organism, or chimeric genes. A "transgene" is a gene that has been introduced into the genome by a transformation procedure.

"Coding sequence" refers to a nucleotide sequence that codes for a specific amino acid sequence. "Regulatory sequences" refer to nucleotide sequences located upstream (5' non-coding sequences), within, or downstream (3' non-coding sequences) of a coding sequence, and which influence the transcription, RNA processing or stability, or translation of the associated coding sequence. Regulatory sequences may include promoters, translation leader sequences, introns, and polyadenylation recognition sequences.

"Promoter" refers to a nucleotide sequence capable of controlling the expression of a coding sequence or functional RNA. In general, a coding sequence is located 3' to a promoter sequence. The promoter sequence consists of proximal and more distal upstream elements, the latter elements often referred to as enhancers. Accordingly, an "enhancer" is a nucleotide sequence which can stimulate promoter activity and may be an innate element of the promoter or a heterologous element inserted to enhance the level or tissue-specificity of a promoter. Promoters may be derived in their entirety from a native gene, or be composed of different elements derived from different promoters found in nature, or even comprise synthetic nucleotide segments. It is understood by those skilled in the art that different promoters may direct the expression of a gene in different tissues or cell types, or at

different stages of development, or in response to different environmental conditions. Promoters which cause a nucleic acid fragment to be expressed in most cell types at most times are commonly referred to as "constitutive promoters". New promoters of various types useful in plant cells are constantly being discovered; numerous examples may be found in the compilation by Okamuro and Goldberg (1989) *Biochemistry of Plants 15*:1-82. It is further recognized that since in most cases the exact boundaries of regulatory sequences have not been completely defined, nucleic acid fragments of different lengths may have identical promoter activity.

The "translation leader sequence" refers to a nucleotide sequence located between the promoter sequence of a gene and the coding sequence. The translation leader sequence is present in the fully processed mRNA upstream of the translation start sequence. The translation leader sequence may affect processing of the primary transcript to mRNA, mRNA stability or translation efficiency. Examples of translation leader sequences have been described (Turner and Foster (1995) *Mol. Biotechnol. 3*:225-236).

The "3' non-coding sequences" refer to nucleotide sequences located downstream of a coding sequence and include polyadenylation recognition sequences and other sequences encoding regulatory signals capable of affecting mRNA processing or gene expression. The polyadenylation signal is usually characterized by affecting the addition of polyadenylic acid tracts to the 3' end of the mRNA precursor. The use of different 3' non-coding sequences is exemplified by Ingelbrecht et al. (1989) *Plant Cell 1*:671-680.

"RNA transcript" refers to the product resulting from RNA polymerase-catalyzed transcription of a DNA sequence. When the RNA transcript is a perfect complementary copy of the DNA sequence, it is referred to as the primary transcript or it may be a RNA sequence derived from posttranscriptional processing of the primary transcript and is referred to as the mature RNA. "Messenger RNA (mRNA)" refers to the RNA that is without introns and that can be translated into polypeptide by the cell. "cDNA" refers to a double-stranded DNA that is complementary to and derived from mRNA. "Sense" RNA refers to an RNA transcript that includes the mRNA and so can be translated into a polypeptide by the cell. "Antisense RNA" refers to an RNA transcript that is complementary to all or part of a target primary transcript or mRNA and that blocks the expression of a target gene (see U.S. Patent No. 5,107,065, incorporated herein by reference). The complementarity of an antisense RNA may be with any part of the specific nucleotide sequence, i.e., at the 5' non-coding sequence, 3' non-coding sequence, introns, or the coding sequence. "Functional RNA" refers to sense RNA, antisense RNA, ribozyme RNA, or other RNA that may not be translated but yet has an effect on cellular processes.

The term "operably linked" refers to the association of two or more nucleic acid fragments on a single nucleic acid fragment so that the function of one is affected by the other. For example, a promoter is operably linked with a coding sequence when it is capable

of affecting the expression of that coding sequence (i.e., that the coding sequence is under the transcriptional control of the promoter). Coding sequences can be operably linked to regulatory sequences in sense or antisense orientation.

The term "expression", as used herein, refers to the transcription and stable accumulation of sense (mRNA) or antisense RNA derived from the nucleic acid fragment of the invention. Expression may also refer to translation of mRNA into a polypeptide. "Antisense inhibition" refers to the production of antisense RNA transcripts capable of suppressing the expression of the target protein. "Overexpression" refers to the production of a gene product in transgenic organisms that exceeds levels of production in normal or non-transformed organisms. "Co-suppression" refers to the production of sense RNA transcripts capable of suppressing the expression of identical or substantially similar foreign or endogenous genes (U.S. Patent No. 5,231,020, incorporated herein by reference).

"Altered levels" refers to the production of gene product(s) in transgenic organisms in amounts or proportions that differ from that of normal or non-transformed organisms.

"Mature" protein refers to a post-translationally processed polypeptide; i.e., one from which any pre- or propeptides present in the primary translation product have been removed. "Precursor" protein refers to the primary product of translation of mRNA; i.e., with pre- and propeptides still present. Pre- and propeptides may be but are not limited to intracellular localization signals.

A "chloroplast transit peptide" is an amino acid sequence which is translated in conjunction with a protein and directs the protein to the chloroplast or other plastid types present in the cell in which the protein is made. "Chloroplast transit sequence" refers to a nucleotide sequence that encodes a chloroplast transit peptide. A "signal peptide" is an amino acid sequence which is translated in conjunction with a protein and directs the protein to the secretory system (Chrispeels (1991) *Ann. Rev. Plant Phys. Plant Mol. Biol. 42*:21-53). If the protein is to be directed to a vacuole, a vacuolar targeting signal (*supra*) can further be added, or if to the endoplasmic reticulum, an endoplasmic reticulum retention signal (*supra*) may be added. If the protein is to be directed to the nucleus, any signal peptide present should be removed and instead a nuclear localization signal included (Raikhel (1992) *Plant Phys. 100*:1627-1632).

"Transformation" refers to the transfer of a nucleic acid fragment into the genome of a host organism, resulting in genetically stable inheritance. Host organisms containing the transformed nucleic acid fragments are referred to as "transgenic" organisms. Examples of methods of plant transformation include *Agrobacterium*-mediated transformation (De Blaere et al. (1987) *Meth. Enzymol. 143*:277) and particle-accelerated or "gene gun" transformation technology (Klein et al. (1987) *Nature (London) 327*:70-73; U.S. Patent No. 4,945,050, incorporated herein by reference).

Standard recombinant DNA and molecular cloning techniques used herein are well known in the art and are described more fully in Sambrook et al. *Molecular Cloning: A Laboratory Manual*; Cold Spring Harbor Laboratory Press: Cold Spring Harbor, 1989 (hereinafter "Maniatis").

Nucleic acid fragments encoding at least a portion of several aminoacyl-tRNA synthetases have been isolated and identified by comparison of random plant cDNA sequences to public databases containing nucleotide and protein sequences using the BLAST algorithms well known to those skilled in the art. The nucleic acid fragments of the instant invention may be used to isolate cDNAs and genes encoding homologous proteins from the same or other plant species. Isolation of homologous genes using sequence-dependent protocols is well known in the art. Examples of sequence-dependent protocols include, but are not limited to, methods of nucleic acid hybridization, and methods of DNA and RNA amplification as exemplified by various uses of nucleic acid amplification technologies (e.g., polymerase chain reaction, ligase chain reaction).

For example, genes encoding other arginyl-tRNA synthetase, glutamyl-tRNA synthetase or histidyl-tRNA synthetase polypeptides, either as cDNAs or genomic DNAs, could be isolated directly by using all or a portion of the instant nucleic acid fragments as DNA hybridization probes to screen libraries from any desired plant employing methodology well known to those skilled in the art. Specific oligonucleotide probes based upon the instant nucleic acid sequences can be designed and synthesized by methods known in the art (Maniatis). Moreover, the entire sequences can be used directly to synthesize DNA probes by methods known to the skilled artisan such as random primer DNA labeling, nick translation, or end-labeling techniques, or RNA probes using available *in vitro* transcription systems. In addition, specific primers can be designed and used to amplify a part or all of the instant sequences. The resulting amplification products can be labeled directly during amplification reactions or labeled after amplification reactions, and used as probes to isolate full length cDNA or genomic fragments under conditions of appropriate stringency.

In addition, two short segments of the instant nucleic acid fragments may be used in polymerase chain reaction protocols to amplify longer nucleic acid fragments encoding homologous genes from DNA or RNA. The polymerase chain reaction may also be performed on a library of cloned nucleic acid fragments wherein the sequence of one primer is derived from the instant nucleic acid fragments, and the sequence of the other primer takes advantage of the presence of the polyadenylic acid tracts to the 3' end of the mRNA precursor encoding plant genes. Alternatively, the second primer sequence may be based upon sequences derived from the cloning vector. For example, the skilled artisan can follow the RACE protocol (Frohman et al. (1988) *Proc. Natl. Acad. Sci. USA 85*:8998-9002) to generate cDNAs by using PCR to amplify copies of the region between a single point in the

transcript and the 3' or 5' end. Primers oriented in the 3' and 5' directions can be designed from the instant sequences. Using commercially available 3' RACE or 5' RACE systems (BRL), specific 3' or 5' cDNA fragments can be isolated (Ohara et al. (1989) *Proc. Natl. Acad. Sci. USA 86*:5673-5677; Loh et al. (1989) *Science 243*:217-220). Products generated by the 3' and 5' RACE procedures can be combined to generate full-length cDNAs (Frohman and Martin (1989) *Techniques 1*:165). Consequently, a polynucleotide comprising a nucleotide sequence of at least one of 60 (preferably one of at least 40, most preferably one of at least 30) contiguous nucleotides derived from a nucleotide sequence selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37 and the complement of such nucleotide sequences may be used in such methods to obtain a nucleic acid fragment encoding a substantial portion of an amino acid sequence of a polypeptide. The present invention relates to a method of obtaining a nucleic acid fragment encoding a substantial portion of a polypeptide of a gene (such as arginyl-tRNA synthetase, glutamyl-tRNA synthetase or histidyl-tRNA synthetase) preferably a substantial portion of a plant polypeptide of a gene, comprising the steps of: synthesizing an oligonucleotide primer comprising a nucleotide sequence of at least one of 60 (preferably at least one of 40, most preferably at least one of 30) contiguous nucleotides derived from a nucleotide sequence selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37 and the complement of such nucleotide sequences; and amplifying a nucleic acid fragment (preferably a cDNA inserted in a cloning vector) using the oligonucleotide primer. The amplified nucleic acid fragment preferably will encode a portion of a polypeptide.

Availability of the instant nucleotide and deduced amino acid sequences facilitates immunological screening of cDNA expression libraries. Synthetic peptides representing portions of the instant amino acid sequences may be synthesized. These peptides can be used to immunize animals to produce polyclonal or monoclonal antibodies with specificity for peptides or proteins comprising the amino acid sequences. These antibodies can be then be used to screen cDNA expression libraries to isolate full-length cDNA clones of interest (Lerner (1984) *Adv. Immunol. 36*:1-34; Maniatis).

The nucleic acid fragments of the instant invention may be used to create transgenic plants in which the disclosed polypeptides are present at higher or lower levels than normal or in cell types or developmental stages in which they are not normally found. This would have the effect of altering the level of in those cells.

Overexpression of the proteins of the instant invention may be accomplished by first constructing a chimeric gene in which the coding region is operably linked to a promoter capable of directing expression of a gene in the desired tissues at the desired stage of development. For reasons of convenience, the chimeric gene may comprise promoter sequences and translation leader sequences derived from the same genes. 3' Non-coding

sequences encoding transcription termination signals may also be provided. The instant chimeric gene may also comprise one or more introns in order to facilitate gene expression.

Plasmid vectors comprising the instant chimeric gene can then be constructed. The choice of plasmid vector is dependent upon the method that will be used to transform host plants. The skilled artisan is well aware of the genetic elements that must be present on the plasmid vector in order to successfully transform, select and propagate host cells containing the chimeric gene. The skilled artisan will also recognize that different independent transformation events will result in different levels and patterns of expression (Jones et al. (1985) *EMBO J. 4*:2411-2418; De Almeida et al. (1989) *Mol. Gen. Genetics 218*:78-86), and thus that multiple events must be screened in order to obtain lines displaying the desired expression level and pattern. Such screening may be accomplished by Southern analysis of DNA, Northern analysis of mRNA expression, Western analysis of protein expression, or phenotypic analysis.

For some applications it may be useful to direct the instant polypeptides to different cellular compartments, or to facilitate its secretion from the cell. It is thus envisioned that the chimeric gene described above may be further supplemented by altering the coding sequence to encode the instant polypeptides with appropriate intracellular targeting sequences such as transit sequences (Keegstra (1989) *Cell 56*:247-253), signal sequences or sequences encoding endoplasmic reticulum localization (Chrispeels (1991) *Ann. Rev. Plant Phys. Plant Mol. Biol. 42*:21-53), or nuclear localization signals (Raikhel (1992) *Plant Phys.100*:1627-1632) added and/or with targeting sequences that are already present removed. While the references cited give examples of each of these, the list is not exhaustive and more targeting signals of utility may be discovered in the future.

It may also be desirable to reduce or eliminate expression of genes encoding the instant polypeptides in plants for some applications. In order to accomplish this, a chimeric gene designed for co-suppression of the instant polypeptide can be constructed by linking a gene or gene fragment encoding that polypeptide to plant promoter sequences. Alternatively, a chimeric gene designed to express antisense RNA for all or part of the instant nucleic acid fragment can be constructed by linking the gene or gene fragment in reverse orientation to plant promoter sequences. Either the co-suppression or antisense chimeric genes could be introduced into plants via transformation wherein expression of the corresponding endogenous genes are reduced or eliminated.

Molecular genetic solutions to the generation of plants with altered gene expression have a decided advantage over more traditional plant breeding approaches. Changes in plant phenotypes can be produced by specifically inhibiting expression of one or more genes by antisense inhibition or cosuppression (U.S. Patent Nos. 5,190,931, 5,107,065 and 5,283,323). An antisense or cosuppression construct would act as a dominant negative regulator of gene activity. While conventional mutations can yield negative regulation of

gene activity these effects are most likely recessive. The dominant negative regulation available with a transgenic approach may be advantageous from a breeding perspective. In addition, the ability to restrict the expression of specific phenotype to the reproductive tissues of the plant by the use of tissue specific promoters may confer agronomic advantages relative to conventional mutations which may have an effect in all tissues in which a mutant gene is ordinarily expressed.

The person skilled in the art will know that special considerations are associated with the use of antisense or cosuppression technologies in order to reduce expression of particular genes. For example, the proper level of expression of sense or antisense genes may require the use of different chimeric genes utilizing different regulatory elements known to the skilled artisan. Once transgenic plants are obtained by one of the methods described above, it will be necessary to screen individual transgenics for those that most effectively display the desired phenotype. Accordingly, the skilled artisan will develop methods for screening large numbers of transformants. The nature of these screens will generally be chosen on practical grounds, and is not an inherent part of the invention. For example, one can screen by looking for changes in gene expression by using antibodies specific for the protein encoded by the gene being suppressed, or one could establish assays that specifically measure enzyme activity. A preferred method will be one which allows large numbers of samples to be processed rapidly, since it will be expected that a large number of transformants will be negative for the desired phenotype.

The instant polypeptides (or portions thereof) may be produced in heterologous host cells, particularly in the cells of microbial hosts, and can be used to prepare antibodies to the these proteins by methods well known to those skilled in the art. The antibodies are useful for detecting the polypeptides of the instant invention *in situ* in cells or *in vitro* in cell extracts. Preferred heterologous host cells for production of the instant polypeptides are microbial hosts. Microbial expression systems and expression vectors containing regulatory sequences that direct high level expression of foreign proteins are well known to those skilled in the art. Any of these could be used to construct a chimeric gene for production of the instant polypeptides. This chimeric gene could then be introduced into appropriate microorganisms via transformation to provide high level expression of the encoded aminoacyl-tRNA synthetase. An example of a vector for high level expression of the instant polypeptides in a bacterial host is provided (Example 8).

Additionally, the instant polypeptides can be used as a targets to facilitate design and/or identification of inhibitors of those enzymes that may be useful as herbicides. This is desirable because the polypeptides described herein catalyze various steps in aminoacyl-tRNA biosynthesis. Accordingly, inhibition of the activity of one or more of the enzymes described herein could lead to inhibition of plant growth. Thus, the instant polypeptides could be appropriate for new herbicide discovery and design.

All or a substantial portion of the nucleic acid fragments of the instant invention may also be used as probes for genetically and physically mapping the genes that they are a part of, and as markers for traits linked to those genes. Such information may be useful in plant breeding in order to develop lines with desired phenotypes. For example, the instant nucleic acid fragments may be used as restriction fragment length polymorphism (RFLP) markers. Southern blots (Maniatis) of restriction-digested plant genomic DNA may be probed with the nucleic acid fragments of the instant invention. The resulting banding patterns may then be subjected to genetic analyses using computer programs such as MapMaker (Lander et al. (1987) *Genomics 1*:174-181) in order to construct a genetic map. In addition, the nucleic acid fragments of the instant invention may be used to probe Southern blots containing restriction endonuclease-treated genomic DNAs of a set of individuals representing parent and progeny of a defined genetic cross. Segregation of the DNA polymorphisms is noted and used to calculate the position of the instant nucleic acid sequence in the genetic map previously obtained using this population (Botstein et al. (1980) *Am. J. Hum. Genet. 32*:314-331).

The production and use of plant gene-derived probes for use in genetic mapping is described in Bernatzky and Tanksley (1986) *Plant Mol. Biol. Reporter 4*:37-41. Numerous publications describe genetic mapping of specific cDNA clones using the methodology outlined above or variations thereof. For example, F2 intercross populations, backcross populations, randomly mated populations, near isogenic lines, and other sets of individuals may be used for mapping. Such methodologies are well known to those skilled in the art.

Nucleic acid probes derived from the instant nucleic acid sequences may also be used for physical mapping (i.e., placement of sequences on physical maps; *see* Hoheisel et al. In: *Nonmammalian Genomic Analysis: A Practical Guide*, Academic press 1996, pp. 319-346, and references cited therein).

In another embodiment, nucleic acid probes derived from the instant nucleic acid sequences may be used in direct fluorescence *in situ* hybridization (FISH) mapping (Trask (1991) *Trends Genet. 7*:149-154). Although current methods of FISH mapping favor use of large clones (several to several hundred KB; see Laan et al. (1995) *Genome Res. 5*:13-20), improvements in sensitivity may allow performance of FISH mapping using shorter probes.

A variety of nucleic acid amplification-based methods of genetic and physical mapping may be carried out using the instant nucleic acid sequences. Examples include allele-specific amplification (Kazazian (1989) *J. Lab. Clin. Med. 11*:95-96), polymorphism of PCR-amplified fragments (CAPS; Sheffield et al. (1993) *Genomics 16*:325-332), allele-specific ligation (Landegren et al. (1988) *Science 241*:1077-1080), nucleotide extension reactions (Sokolov (1990) *Nucleic Acid Res. 18*:3671), Radiation Hybrid Mapping (Walter et al. (1997) *Nat. Genet. 7*:22-28) and Happy Mapping (Dear and Cook (1989) *Nucleic Acid Res. 17*:6795-6807). For these methods, the sequence of a nucleic acid fragment is used to

design and produce primer pairs for use in the amplification reaction or in primer extension reactions. The design of such primers is well known to those skilled in the art. In methods employing PCR-based genetic mapping, it may be necessary to identify DNA sequence differences between the parents of the mapping cross in the region corresponding to the instant nucleic acid sequence. This, however, is generally not necessary for mapping methods.

Loss of function mutant phenotypes may be identified for the instant cDNA clones either by targeted gene disruption protocols or by identifying specific mutants for these genes contained in a maize population carrying mutations in all possible genes (Ballinger and Benzer (1989) *Proc. Natl. Acad. Sci USA 86*:9402-9406; Koes et al. (1995) *Proc. Natl. Acad. Sci USA 92*:8149-8153; Bensen et al. (1995) *Plant Cell 7*:75-84). The latter approach may be accomplished in two ways. First, short segments of the instant nucleic acid fragments may be used in polymerase chain reaction protocols in conjunction with a mutation tag sequence primer on DNAs prepared from a population of plants in which Mutator transposons or some other mutation-causing DNA element has been introduced (see Bensen, *supra*). The amplification of a specific DNA fragment with these primers indicates the insertion of the mutation tag element in or near the plant gene encoding the instant polypeptides. Alternatively, the instant nucleic acid fragment may be used as a hybridization probe against PCR amplification products generated from the mutation population using the mutation tag sequence primer in conjunction with an arbitrary genomic site primer, such as that for a restriction enzyme site-anchored synthetic adaptor. With either method, a plant containing a mutation in the endogenous gene encoding the instant polypeptides can be identified and obtained. This mutant plant can then be used to determine or confirm the natural function of the instant polypeptides disclosed herein.

EXAMPLES

The present invention is further defined in the following Examples, in which all parts and percentages are by weight and degrees are Celsius, unless otherwise stated. It should be understood that these Examples, while indicating preferred embodiments of the invention, are given by way of illustration only. From the above discussion and these Examples, one skilled in the art can ascertain the essential characteristics of this invention, and without departing from the spirit and scope thereof, can make various changes and modifications of the invention to adapt it to various usages and conditions.

EXAMPLE 1

Composition of cDNA Libraries; Isolation and Sequencing of cDNA Clones

cDNA libraries representing mRNAs from various corn, rice, soybean and wheat tissues were prepared. The characteristics of the libraries are described below.

TABLE 2

cDNA Libraries from Corn, Rice, Soybean and Wheat

Library	Tissue	Clone
cpc1c	Corn pooled BMS treated with chemicals related to cGMP**	cpc1c.pk001.d11
p0010	Corn log phase suspension cells treated with A23187® to induce mass apoptosis****	p0010.cbpcp10r
p0102	Corn early meiosis tassels*	p0102.cerbb73r
rl0n	Rice 15 day old leaf*	rl0n.pk086.p16
rlr2	Rice leaf 15 days after germination, 2 hours after infection of strain *Magaporthe grisea* 4360-R-62 (AVR2-YAMO)	rlr2.pk0032.f2
sdc5c		sdc5c.pk0002.e11
sdp4c		sdp4c.pk007.c7
sgs1c	Soybean seeds 4 hours after germination	sgs1c.pk001.k12
		sgs1c.pk004.e20
ssm	Soybean shoot meristem	ssm.pk0012.d9
		ssm.pk0026.b10
wlk1	Wheat seedlings 1 hour after inoculation with *Erysiphe graminis f. sp tritici* and treatment with herbicide***	wlk1.pk0007.f5
wlm96	Wheat Seedlings 96 hours after inoculation with *Erysiphe graminis f. sp tritici*	wlm96.pk055.g5
wr1	Wheat root from 7 day old seedling	wr1.pk0079.d1

*These libraries were normalized essentially as described in U.S. Patent No. 5,482,845, incorporated herein by reference.

**Corn tissues were individually treated with one of the following then pooled; Suramin, MAS7, dipyryridamole, zaprinast, 8-bromo-cGMPtrequinsin HCL, Compound 48/80 all of which are commercially available from Calbiochem-Noavbiochem Corp.

***Application of 6-iodo-2-propoxy-3-propyl-4(3*H*)-quinazolinone; synthesis and methods of using this compound are described in USSN 08/545,827, incorporated herein by reference.

****A23187® is commercially available from several vendors including Calbiochem

cDNA libraries may be prepared by any one of many methods available. For example, the cDNAs may be introduced into plasmid vectors by first preparing the cDNA libraries in Uni-ZAP™ XR vectors according to the manufacturer's protocol (Stratagene Cloning Systems, La Jolla, CA). The Uni-ZAP™ XR libraries are converted into plasmid libraries according to the protocol provided by Stratagene. Upon conversion, cDNA inserts will be contained in the plasmid vector pBluescript. In addition, the cDNAs may be introduced directly into precut Bluescript II SK(+) vectors (Stratagene) using T4 DNA ligase (New England Biolabs), followed by transfection into DH10B cells according to the manufacturer's protocol (GIBCO BRL Products). Once the cDNA inserts are in plasmid vectors, plasmid DNAs are prepared from randomly picked bacterial colonies containing

recombinant pBluescript plasmids, or the insert cDNA sequences are amplified via polymerase chain reaction using primers specific for vector sequences flanking the inserted cDNA sequences. Amplified insert DNAs or plasmid DNAs are sequenced in dye-primer sequencing reactions to generate partial cDNA sequences (expressed sequence tags or "ESTs"; see Adams et al., (1991) *Science 252*:1651-1656). The resulting ESTs are analyzed using a Perkin Elmer Model 377 fluorescent sequencer.

EXAMPLE 2

Identification of cDNA Clones

cDNA clones encoding aminoacyl-tRNA synthetases were identified by conducting BLAST (Basic Local Alignment Search Tool; Altschul et al. (1993) *J. Mol. Biol. 215*:403-410; see also www.ncbi.nlm.nih.gov/BLAST/) searches for similarity to sequences contained in the BLAST "nr" database (comprising all non-redundant GenBank CDS translations, sequences derived from the 3-dimensional structure Brookhaven Protein Data Bank, the last major release of the SWISS-PROT protein sequence database, EMBL, and DDBJ databases). The cDNA sequences obtained in Example 1 were analyzed for similarity to all publicly available DNA sequences contained in the "nr" database using the BLASTN algorithm provided by the National Center for Biotechnology Information (NCBI). The DNA sequences were translated in all reading frames and compared for similarity to all publicly available protein sequences contained in the "nr" database using the BLASTX algorithm (Gish and States (1993) *Nat. Genet. 3*:266-272) provided by the NCBI. For convenience, the P-value (probability) of observing a match of a cDNA sequence to a sequence contained in the searched databases merely by chance as calculated by BLAST are reported herein as "pLog" values, which represent the negative of the logarithm of the reported P-value. Accordingly, the greater the pLog value, the greater the likelihood that the cDNA sequence and the BLAST "hit" represent homologous proteins.

EXAMPLE 3

Characterization of cDNA Clones Encoding Arginyl-tRNA Synthetase

The BLASTX search using the EST sequences from clones listed in Table 3 revealed similarity of the polypeptides encoded by the cDNAs to arginyl-tRNA synthetase from *Arabidopsis thaliana* (NCBI Identifier No. gi 2632105). Shown in Table 3 are the BLAST results for individual ESTs ("EST"), the sequences of the entire cDNA inserts comprising the indicated cDNA clones ("FIS"), contigs assembled from two or more ESTs ("Contig"), contigs assembled from an FIS and one or more ESTs ("Contig*"), or sequences encoding the entire protein derived from an FIS, a contig, or an FIS and PCR ("CGS"):

TABLE 3

BLAST Results for Sequences Encoding Polypeptides Homologous to *Arabidopsis thaliana* Arginyl-tRNA Synthetase

Clone	Status	BLAST pLog Score to gi 2632105
cpc1c.pk001.d11	(FIS)	146.00
rl0n.pk086.p16	(FIS)	>254.00
ssm.pk0026.b10	(FIS)	129.00
wlk1.pk0007.f5	(FIS)	102.00

The data in Table 4 represents a calculation of the percent identity of the amino acid sequences set forth in SEQ ID NOs:2, 4, 6 and 8 and the *Arabidopsis thaliana* sequence.

TABLE 4

Percent Identity of Amino Acid Sequences Deduced From the Nucleotide Sequences of cDNA Clones Encoding Polypeptides Homologous to *Arabidopsis thaliana* Arginyl-tRNA Synthetase

SEQ ID NO.	Percent Identity to gi 2632105
2	75%
4	66%
6	74%
8	73%

Sequence alignments and percent identity calculations were performed using the Megalign program of the LASERGENE bioinformatics computing suite (DNASTAR Inc., Madison, WI). Multiple alignment of the sequences was performed using the Clustal method of alignment (Higgins and Sharp (1989) *CABIOS. 5*:151-153) with the default parameters (GAP PENALTY=10, GAP LENGTH PENALTY=10). Default parameters for pairwise alignments using the Clustal method were KTUPLE 1, GAP PENALTY=3, WINDOW=5 and DIAGONALS SAVED=5. Sequence alignments and BLAST scores and probabilities indicate that the nucleic acid fragments comprising the instant cDNA clones encode a substantial portion of a arginyl-tRNA synthetase. These sequences represent the first corn, rice, soybean and wheat sequences encoding arginyl-tRNA synthetase.

EXAMPLE 4

Characterization of cDNA Clones Encoding Glutamyl-tRNA Synthetase

The BLASTX search using the EST sequences from clones listed in Table 5 revealed similarity of the polypeptides encoded by the cDNAs to glutamyl-tRNA synthetase from *Arabidopsis thaliana* (NCBI Identifier No. gi 3435196), *Hordum vulgare* (NCBI Identifier No. gi 2500980), *Nicotina tabacum* (NCBI Identifier No. gi 2500981) and *Saccharomyces*

cerevisiae (NCBI Identifier No. gi 2507428). Shown in Table 5 are the BLAST results for individual ESTs ("EST"), the sequences of the entire cDNA inserts comprising the indicated cDNA clones ("FIS"), contigs assembled from two or more ESTs ("Contig"), contigs assembled from an FIS and one or more ESTs ("Contig*"), or sequences encoding the entire protein derived from an FIS, a contig, or an FIS and PCR ("CGS"):

TABLE 5

BLAST Results for Sequences Encoding Polypeptides Homologous to *Arabidopsis thaliana*, *Hordum vulgare*, *Nicotina tabacum* and *Saccharomyces cerevisiae* Glutamyl-tRNA Synthetase

Clone	Status	BLAST pLog Score
p0010.cbpcp10r	(CGS)	>254.00 (gi 3435196)
rlr2.pk0032.f2	(CGS)	>254.00 (gi 2500980)
Contig composed of: sdc5c.pk0002.e11 sgs1c.pk001.k12 sgs1c.pk004.e20	Contig	97.40 (gi 2500981)
wlm96.pk055.g5	(EST)	18.30 (gi 2507428)

The data in Table 6 represents a calculation of the percent identity of the amino acid sequences set forth in SEQ ID NOs:10, 12, 14 and 16 and the *Arabidopsis thaliana*, *Hordum vulgare*, *Nicotina tabacum* and *Saccharomyces cerevisiae* sequences.

TABLE 6

Percent Identity of Amino Acid Sequences Deduced From the Nucleotide Sequences of cDNA Clones Encoding Polypeptides Homologous to *Arabidopsis thaliana*, *Hordum vulgare*, *Nicotina tabacum* and *Saccharomyces cerevisiae* Glutamyl-tRNA Synthetase

SEQ ID NO.	Percent Identity to
10	67% (gi 3435196)
12	81% (gi 2500980)
14	87% (gi 2500981)
16	54% (gi 2507428)

Sequence alignments and percent identity calculations were performed using the Megalign program of the LASERGENE bioinformatics computing suite (DNASTAR Inc., Madison, WI). Multiple alignment of the sequences was performed using the Clustal method of alignment (Higgins and Sharp (1989) *CABIOS. 5*:151-153) with the default parameters (GAP PENALTY=10, GAP LENGTH PENALTY=10). Default parameters for pairwise alignments using the Clustal method were KTUPLE 1, GAP PENALTY=3, WINDOW=5 and DIAGONALS SAVED=5. Sequence alignments and BLAST scores and probabilities indicate that the nucleic acid fragments comprising the instant cDNA clones

encode a substantial portion of a glutamyl-tRNA synthetase. These sequences represent the first corn, rice, soybean and wheat sequences encoding glutamyl-tRNA synthetase.

EXAMPLE 5

Characterization of cDNA Clones Encoding Histidyl-tRNA Synthetase

The BLASTX search using the EST sequences from clones listed in Table 7 revealed similarity of the polypeptides encoded by the cDNAs to histidyl-tRNA synthetase from *Oryza sativa* (NCBI Identifier No. gi 3915070) and *Arabidopsis thaliana* (NCBI Identifier No. gi 3659909). Shown in Table 7 are the BLAST results for individual ESTs ("EST"), the sequences of the entire cDNA inserts comprising the indicated cDNA clones ("FIS"), contigs assembled from two or more ESTs ("Contig"), contigs assembled from an FIS and one or more ESTs ("Contig*"), or sequences encoding the entire protein derived from an FIS, a contig, or an FIS and PCR ("CGS"):

TABLE 7

BLAST Results for Sequences Encoding Polypeptides Homologous to *Oryza sativa* and *Arabidopsis thaliana* Histidyl-tRNA Synthetase

Clone	Status	BLAST pLog Score
p0102.cerbb73r	(CGS)	>254.00 (gi 3915070)
Contig composed of: sdp4c.pk007.c7 ssm.pk0012.d9	Contig	84.22 (gi 3659909)
wr1.pk0079.d1	(FIS)	98.30 (gi 3659909)

The data in Table 8 represents a calculation of the percent identity of the amino acid sequences set forth in SEQ ID NOs:18, 20 and 22 and the *Oryza sativa* and *Arabidopsis thaliana* sequences.

TABLE 8

Percent Identity of Amino Acid Sequences Deduced From the Nucleotide Sequences of cDNA Clones Encoding Polypeptides Homologous to *Oryza sativa* and *Arabidopsis thaliana* Histidyl-tRNA Synthetase

SEQ ID NO.	Percent Identity to
18	87%
20	67%
22	67%

Sequence alignments and percent identity calculations were performed using the Megalign program of the LASERGENE bioinformatics computing suite (DNASTAR Inc., Madison, WI). Multiple alignment of the sequences was performed using the Clustal method of alignment (Higgins and Sharp (1989) *CABIOS. 5*:151-153) with the default

parameters (GAP PENALTY=10, GAP LENGTH PENALTY=10). Default parameters for pairwise alignments using the Clustal method were KTUPLE 1, GAP PENALTY=3, WINDOW=5 and DIAGONALS SAVED=5. Sequence alignments and BLAST scores and probabilities indicate that the nucleic acid fragments comprising the instant cDNA clones encode a substantial portion of a histidyl-tRNA synthetase. These sequences represent the first corn, soybean and wheat sequences encoding histidyl-tRNA synthetase.

EXAMPLE 6

Expression of Chimeric Genes in Monocot Cells

A chimeric gene comprising a cDNA encoding the instant polypeptides in sense orientation with respect to the maize 27 kD zein promoter that is located 5' to the cDNA fragment, and the 10 kD zein 3' end that is located 3' to the cDNA fragment, can be constructed. The cDNA fragment of this gene may be generated by polymerase chain reaction (PCR) of the cDNA clone using appropriate oligonucleotide primers. Cloning sites (NcoI or SmaI) can be incorporated into the oligonucleotides to provide proper orientation of the DNA fragment when inserted into the digested vector pML103 as described below. Amplification is then performed in a standard PCR. The amplified DNA is then digested with restriction enzymes NcoI and SmaI and fractionated on an agarose gel. The appropriate band can be isolated from the gel and combined with a 4.9 kb NcoI-SmaI fragment of the plasmid pML103. Plasmid pML103 has been deposited under the terms of the Budapest Treaty at ATCC (American Type Culture Collection, 10801 University Blvd., Manassas, VA 20110-2209), and bears accession number ATCC 97366. The DNA segment from pML103 contains a 1.05 kb SalI-NcoI promoter fragment of the maize 27 kD zein gene and a 0.96 kb SmaI-SalI fragment from the 3' end of the maize 10 kD zein gene in the vector pGem9Zf(+) (Promega). Vector and insert DNA can be ligated at 15°C overnight, essentially as described (Maniatis). The ligated DNA may then be used to transform *E. coli* XL1-Blue (Epicurian Coli XL-1 Blue™; Stratagene). Bacterial transformants can be screened by restriction enzyme digestion of plasmid DNA and limited nucleotide sequence analysis using the dideoxy chain termination method (Sequenase™ DNA Sequencing Kit; U.S. Biochemical). The resulting plasmid construct would comprise a chimeric gene encoding, in the 5' to 3' direction, the maize 27 kD zein promoter, a cDNA fragment encoding the instant polypeptides, and the 10 kD zein 3' region.

The chimeric gene described above can then be introduced into corn cells by the following procedure. Immature corn embryos can be dissected from developing caryopses derived from crosses of the inbred corn lines H99 and LH132. The embryos are isolated 10 to 11 days after pollination when they are 1.0 to 1.5 mm long. The embryos are then placed with the axis-side facing down and in contact with agarose-solidified N6 medium (Chu et al. (1975) *Sci. Sin. Peking* 18:659-668). The embryos are kept in the dark at 27°C. Friable embryogenic callus consisting of undifferentiated masses of cells with somatic

proembryoids and embryoids borne on suspensor structures proliferates from the scutellum of these immature embryos. The embryogenic callus isolated from the primary explant can be cultured on N6 medium and sub-cultured on this medium every 2 to 3 weeks.

The plasmid, p35S/Ac (obtained from Dr. Peter Eckes, Hoechst Ag, Frankfurt, Germany) may be used in transformation experiments in order to provide for a selectable marker. This plasmid contains the *Pat* gene (see European Patent Publication 0 242 236) which encodes phosphinothricin acetyl transferase (PAT). The enzyme PAT confers resistance to herbicidal glutamine synthetase inhibitors such as phosphinothricin. The *pat* gene in p35S/Ac is under the control of the 35S promoter from Cauliflower Mosaic Virus (Odell et al. (1985) *Nature* 313:810-812) and the 3' region of the nopaline synthase gene from the T-DNA of the Ti plasmid of *Agrobacterium tumefaciens*.

The particle bombardment method (Klein et al. (1987) *Nature* 327:70-73) may be used to transfer genes to the callus culture cells. According to this method, gold particles (1 μm in diameter) are coated with DNA using the following technique. Ten μg of plasmid DNAs are added to 50 μL of a suspension of gold particles (60 mg per mL). Calcium chloride (50 μL of a 2.5 M solution) and spermidine free base (20 μL of a 1.0 M solution) are added to the particles. The suspension is vortexed during the addition of these solutions. After 10 minutes, the tubes are briefly centrifuged (5 sec at 15,000 rpm) and the supernatant removed. The particles are resuspended in 200 μL of absolute ethanol, centrifuged again and the supernatant removed. The ethanol rinse is performed again and the particles resuspended in a final volume of 30 μL of ethanol. An aliquot (5 μL) of the DNA-coated gold particles can be placed in the center of a Kapton™ flying disc (Bio-Rad Labs). The particles are then accelerated into the corn tissue with a Biolistic™ PDS-1000/He (Bio-Rad Instruments, Hercules CA), using a helium pressure of 1000 psi, a gap distance of 0.5 cm and a flying distance of 1.0 cm.

For bombardment, the embryogenic tissue is placed on filter paper over agarose-solidified N6 medium. The tissue is arranged as a thin lawn and covered a circular area of about 5 cm in diameter. The petri dish containing the tissue can be placed in the chamber of the PDS-1000/He approximately 8 cm from the stopping screen. The air in the chamber is then evacuated to a vacuum of 28 inches of Hg. The macrocarrier is accelerated with a helium shock wave using a rupture membrane that bursts when the He pressure in the shock tube reaches 1000 psi.

Seven days after bombardment the tissue can be transferred to N6 medium that contains gluphosinate (2 mg per liter) and lacks casein or proline. The tissue continues to grow slowly on this medium. After an additional 2 weeks the tissue can be transferred to fresh N6 medium containing gluphosinate. After 6 weeks, areas of about 1 cm in diameter of actively growing callus can be identified on some of the plates containing the glufosinate-

supplemented medium. These calli may continue to grow when sub-cultured on the selective medium.

Plants can be regenerated from the transgenic callus by first transferring clusters of tissue to N6 medium supplemented with 0.2 mg per liter of 2,4-D. After two weeks the tissue can be transferred to regeneration medium (Fromm et al. (1990) *Bio/Technology 8*:833-839).

EXAMPLE 7

Expression of Chimeric Genes in Dicot Cells

A seed-specific expression cassette composed of the promoter and transcription terminator from the gene encoding the β subunit of the seed storage protein phaseolin from the bean *Phaseolus vulgaris* (Doyle et al. (1986) *J. Biol. Chem. 261*:9228-9238) can be used for expression of the instant polypeptides in transformed soybean. The phaseolin cassette includes about 500 nucleotides upstream (5') from the translation initiation codon and about 1650 nucleotides downstream (3') from the translation stop codon of phaseolin. Between the 5' and 3' regions are the unique restriction endonuclease sites Nco I (which includes the ATG translation initiation codon), Sma I, Kpn I and Xba I. The entire cassette is flanked by Hind III sites.

The cDNA fragment of this gene may be generated by polymerase chain reaction (PCR) of the cDNA clone using appropriate oligonucleotide primers. Cloning sites can be incorporated into the oligonucleotides to provide proper orientation of the DNA fragment when inserted into the expression vector. Amplification is then performed as described above, and the isolated fragment is inserted into a pUC18 vector carrying the seed expression cassette.

Soybean embryos may then be transformed with the expression vector comprising sequences encoding the instant polypeptides. To induce somatic embryos, cotyledons, 3-5 mm in length dissected from surface sterilized, immature seeds of the soybean cultivar A2872, can be cultured in the light or dark at 26°C on an appropriate agar medium for 6-10 weeks. Somatic embryos which produce secondary embryos are then excised and placed into a suitable liquid medium. After repeated selection for clusters of somatic embryos which multiplied as early, globular staged embryos, the suspensions are maintained as described below.

Soybean embryogenic suspension cultures can maintained in 35 mL liquid media on a rotary shaker, 150 rpm, at 26°C with florescent lights on a 16:8 hour day/night schedule. Cultures are subcultured every two weeks by inoculating approximately 35 mg of tissue into 35 mL of liquid medium.

Soybean embryogenic suspension cultures may then be transformed by the method of particle gun bombardment (Klein et al. (1987) *Nature* (London) *327*:70-73, U.S. Patent

No. 4,945,050). A DuPont Biolistic™ PDS1000/HE instrument (helium retrofit) can be used for these transformations.

A selectable marker gene which can be used to facilitate soybean transformation is a chimeric gene composed of the 35S promoter from Cauliflower Mosaic Virus (Odell et al. (1985) *Nature 313*:810-812), the hygromycin phosphotransferase gene from plasmid pJR225 (from *E. coli*; Gritz et al.(1983) *Gene 25*:179-188) and the 3' region of the nopaline synthase gene from the T-DNA of the Ti plasmid of *Agrobacterium tumefaciens*. The seed expression cassette comprising the phaseolin 5' region, the fragment encoding the instant polypeptides and the phaseolin 3' region can be isolated as a restriction fragment. This fragment can then be inserted into a unique restriction site of the vector carrying the marker gene.

To 50 μL of a 60 mg/mL 1 μm gold particle suspension is added (in order): 5 μL DNA (1 μg/μL), 20 μl spermidine (0.1 M), and 50 μL $CaCl_2$ (2.5 M). The particle preparation is then agitated for three minutes, spun in a microfuge for 10 seconds and the supernatant removed. The DNA-coated particles are then washed once in 400 μL 70% ethanol and resuspended in 40 μL of anhydrous ethanol. The DNA/particle suspension can be sonicated three times for one second each. Five μL of the DNA-coated gold particles are then loaded on each macro carrier disk.

Approximately 300-400 mg of a two-week-old suspension culture is placed in an empty 60x15 mm petri dish and the residual liquid removed from the tissue with a pipette. For each transformation experiment, approximately 5-10 plates of tissue are normally bombarded. Membrane rupture pressure is set at 1100 psi and the chamber is evacuated to a vacuum of 28 inches mercury. The tissue is placed approximately 3.5 inches away from the retaining screen and bombarded three times. Following bombardment, the tissue can be divided in half and placed back into liquid and cultured as described above.

Five to seven days post bombardment, the liquid media may be exchanged with fresh media, and eleven to twelve days post bombardment with fresh media containing 50 mg/mL hygromycin. This selective media can be refreshed weekly. Seven to eight weeks post bombardment, green, transformed tissue may be observed growing from untransformed, necrotic embryogenic clusters. Isolated green tissue is removed and inoculated into individual flasks to generate new, clonally propagated, transformed embryogenic suspension cultures. Each new line may be treated as an independent transformation event. These suspensions can then be subcultured and maintained as clusters of immature embryos or regenerated into whole plants by maturation and germination of individual somatic embryos.

EXAMPLE 8

Expression of Chimeric Genes in Microbial Cells

The cDNAs encoding the instant polypeptides can be inserted into the T7 *E. coli* expression vector pBT430. This vector is a derivative of pET-3a (Rosenberg et al. (1987) *Gene 56*:125-135) which employs the bacteriophage T7 RNA polymerase/T7 promoter

system. Plasmid pBT430 was constructed by first destroying the EcoR I and Hind III sites in pET-3a at their original positions. An oligonucleotide adaptor containing EcoR I and Hind III sites was inserted at the BamH I site of pET-3a. This created pET-3aM with additional unique cloning sites for insertion of genes into the expression vector. Then, the Nde I site at the position of translation initiation was converted to an Nco I site using oligonucleotide-directed mutagenesis. The DNA sequence of pET-3aM in this region, 5'-CATATGG, was converted to 5'-CCCATGG in pBT430.

Plasmid DNA containing a cDNA may be appropriately digested to release a nucleic acid fragment encoding the protein. This fragment may then be purified on a 1% NuSieve GTG™ low melting agarose gel (FMC). Buffer and agarose contain 10 μg/ml ethidium bromide for visualization of the DNA fragment. The fragment can then be purified from the agarose gel by digestion with GELase™ (Epicentre Technologies) according to the manufacturer's instructions, ethanol precipitated, dried and resuspended in 20 μL of water. Appropriate oligonucleotide adapters may be ligated to the fragment using T4 DNA ligase (New England Biolabs, Beverly, MA). The fragment containing the ligated adapters can be purified from the excess adapters using low melting agarose as described above. The vector pBT430 is digested, dephosphorylated with alkaline phosphatase (NEB) and deproteinized with phenol/chloroform as described above. The prepared vector pBT430 and fragment can then be ligated at 16°C for 15 hours followed by transformation into DH5 electrocompetent cells (GIBCO BRL). Transformants can be selected on agar plates containing LB media and 100 μg/mL ampicillin. Transformants containing the gene encoding the instant polypeptides are then screened for the correct orientation with respect to the T7 promoter by restriction enzyme analysis.

For high level expression, a plasmid clone with the cDNA insert in the correct orientation relative to the T7 promoter can be transformed into *E. coli* strain BL21(DE3) (Studier et al. (1986) *J. Mol. Biol. 189*:113-130). Cultures are grown in LB medium containing ampicillin (100 mg/L) at 25°C. At an optical density at 600 nm of approximately 1, IPTG (isopropylthio-β-galactoside, the inducer) can be added to a final concentration of 0.4 mM and incubation can be continued for 3 h at 25°. Cells are then harvested by centrifugation and re-suspended in 50 μL of 50 mM Tris-HCl at pH 8.0 containing 0.1 mM DTT and 0.2 mM phenyl methylsulfonyl fluoride. A small amount of 1 mm glass beads can be added and the mixture sonicated 3 times for about 5 seconds each time with a microprobe sonicator. The mixture is centrifuged and the protein concentration of the supernatant determined. One μg of protein from the soluble fraction of the culture can be separated by SDS-polyacrylamide gel electrophoresis. Gels can be observed for protein bands migrating at the expected molecular weight.

EXAMPLE 9

Evaluating Compounds for Their Ability to Inhibit the Activity of Aminoacyl-tRNA Synthetases

The polypeptides described herein may be produced using any number of methods known to those skilled in the art. Such methods include, but are not limited to, expression in bacteria as described in Example 8, or expression in eukaryotic cell culture, *in planta*, and using viral expression systems in suitably infected organisms or cell lines. The instant polypeptides may be expressed either as mature forms of the proteins as observed *in vivo* or as fusion proteins by covalent attachment to a variety of enzymes, proteins or affinity tags. Common fusion protein partners include glutathione S-transferase ("GST"), thioredoxin ("Trx"), maltose binding protein, and C- and/or N-terminal hexahistidine polypeptide ("$(His)_6$"). The fusion proteins may be engineered with a protease recognition site at the fusion point so that fusion partners can be separated by protease digestion to yield intact mature enzyme. Examples of such proteases include thrombin, enterokinase and factor Xa. However, any protease can be used which specifically cleaves the peptide connecting the fusion protein and the enzyme.

Purification of the instant polypeptides, if desired, may utilize any number of separation technologies familiar to those skilled in the art of protein purification. Examples of such methods include, but are not limited to, homogenization, filtration, centrifugation, heat denaturation, ammonium sulfate precipitation, desalting, pH precipitation, ion exchange chromatography, hydrophobic interaction chromatography and affinity chromatography, wherein the affinity ligand represents a substrate, substrate analog or inhibitor. When the instant polypeptides are expressed as fusion proteins, the purification protocol may include the use of an affinity resin which is specific for the fusion protein tag attached to the expressed enzyme or an affinity resin containing ligands which are specific for the enzyme. For example, the instant polypeptides may be expressed as a fusion protein coupled to the C-terminus of thioredoxin. In addition, a $(His)_6$ peptide may be engineered into the N-terminus of the fused thioredoxin moiety to afford additional opportunities for affinity purification. Other suitable affinity resins could be synthesized by linking the appropriate ligands to any suitable resin such as Sepharose-4B. In an alternate embodiment, a thioredoxin fusion protein may be eluted using dithiothreitol; however, elution may be accomplished using other reagents which interact to displace the thioredoxin from the resin. These reagents include β–mercaptoethanol or other reduced thiol. The eluted fusion protein may be subjected to further purification by traditional means as stated above, if desired. Proteolytic cleavage of the thioredoxin fusion protein and the enzyme may be accomplished after the fusion protein is purified or while the protein is still bound to the ThioBond™ affinity resin or other resin.

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Crude, partially purified or purified enzyme, either alone or as a fusion protein, may be utilized in assays for the evaluation of compounds for their ability to inhibit enzymatic activation of the instant polypeptides disclosed herein. Assays may be conducted under well known experimental conditions which permit optimal enzymatic activity. For example, assays for aminoacyl-tRNA synthetase activity are presented by (Lloyd et al., (1995) *Nucleic Acid Research 23(15)*:2882-2892).

CLAIMS

What is claimed is:

1. An isolated polynucleotide comprising a nucleotide sequence encoding a first polypeptide of at least 240 amino acids that has at least 80% identity based on the Clustal method of alignment when compared to a polypeptide selected from the group consisting of arginyl-tRNA synthetase polypeptides of SEQ ID NOs:2, 4, 6 and 8,

or an isolated polynucleotide comprising the complement of the nucleotide sequence.

2. The isolated polynucleotide of Claim 1, wherein the isolated nucleotide sequence consists of a nucleic acid sequence selected from the group consisting of SEQ ID NOs:1, 3, 5, and 7 that codes for the polypeptide selected from the group consisting of SEQ ID NOs:2, 4, 6, and 8.

3. The isolated polynucleotide of Claim 1 wherein the nucleotide sequence is DNA.

4. The isolated polynucleotide of Claim 1 wherein the nucleotide sequence is RNA.

5. A chimeric gene comprising the isolated polynucleotide of Claim 1 operably linked to suitable regulatory sequences.

6. An isolated host cell comprising the chimeric gene of Claim 5.

7. An isolated host cell comprising an isolated polynucleotide of Claim 1.

8. The isolated host cell of Claim 7 wherein the isolated host is selected from the group consisting of yeast, bacteria, plant, and virus.

9. A virus comprising the isolated polynucleotide of Claim 1.

10. A polypeptide of at least 240 amino acids that has at least 80% identity based on the Clustal method of alignment when compared to a polypeptide selected from the group consisting of SEQ ID NO:2, 4, 6 and 8.

11. An isolated polynucleotide comprising a nucleotide sequence encoding a first polypeptide of at least 205 amino acids that has at least 90% identity based on the Clustal method of alignment when compared to a polypeptide selected from the group consisting of glutamyl-tRNA synthetase polypeptides of SEQ ID NOs:10, 12 and 14,

or an isolated polynucleotide comprising the complement of the nucleotide sequence.

12. The isolated polynucleotide of Claim 11, wherein the isolated nucleotide sequence consists of a nucleic acid sequence selected from the group consisting of SEQ ID NOs:9, 11 and 13 that codes for the polypeptide selected from the group consisting of SEQ ID NOs:10, 12 and 14.

13. The isolated polynucleotide of Claim 11 wherein the isolated polynucleotide is DNA.

14. The isolated polynucleotide of Claim 11 wherein the isolated polynucleotide is RNA.

15. A chimeric gene comprising the isolated polynucleotide of Claim 11 operably linked to suitable regulatory sequences.

16. An isolated host cell comprising the chimeric gene of Claim 15.

17. An isolated host cell comprising an isolated polynucleotide of Claim 11.

18. The isolated host cell of Claim 17 wherein the isolated host selected from the group consisting of yeast, bacteria, plant, and virus.

19. A virus comprising the isolated polynucleotide of Claim 11.

20. A polypeptide of at least 205 amino acids that has at least 90% identity based on the Clustal method of alignment when compared to a polypeptide selected from the group consisting of a glutamyl-tRNA synthetase polypeptide of SEQ ID NOs:10, 12 and 14.

21. An isolated polynucleotide comprising a nucleotide sequence encoding a first polypeptide of at least 79 amino acids that has at least 80% identity based on the Clustal method of alignment when compared to a glutamyl-tRNA synthetase polypeptide of SEQ ID NO:16,

or an isolated polynucleotide comprising the complement of the nucleotide sequence.

22. The isolated polynucleotide of Claim 21, wherein the isolated nucleotide sequence consists of a nucleic acid sequence of SEQ ID NO:15 that codes for the polypeptide of SEQ ID NO:16.

23. The isolated polynucleotide of Claim 21 wherein the isolated polynucleotide is DNA.

24. The isolated polynucleotide of Claim 21 wherein the isolated polynucleotide is RNA.

25. A chimeric gene comprising the isolated polynucleotide of Claim 21 operably linked to suitable regulatory sequences.

26. An isolated host cell comprising the chimeric gene of Claim 25.

27. An isolated host cell comprising an isolated polynucleotide of Claim 21.

28. The isolated host cell of Claim 27 wherein the isolated host is selected from the group consisting of yeast, bacteria, plant, and virus.

29. A virus comprising the isolated polynucleotide of Claim 21.

30. A polypeptide of at least 79 amino acids that has at least 80% identity based on the Clustal method of alignment when compared to a glutamyl-tRNA synthetase polypeptide of SEQ ID NO:16.

31. An isolated polynucleotide comprising a nucleotide sequence encoding a first polypeptide of at least 243 amino acids that has at least 90% identity based on the Clustal method of alignment when compared to a polypeptide selected from the group consisting of histidyl-tRNA synthetase polypeptides of SEQ ID NO:s18, 20 and 22,

or an isolated polynucleotide comprising the complement of the nucleotide sequence.

32. The isolated polynucleotide of Claim 31, wherein the isolated nucleotide sequence consists of a nucleic acid sequence selected from the group consisting of SEQ ID NOs:17, 19 and 21 that codes for the polypeptide selected from the group consisting of SEQ ID NOs:18, 20 and 22.

33. The isolated polynucleotide of Claim 31 wherein the isolated polynucleotide is DNA.

34. The isolated polynucleotide of Claim 31 wherein the isolated polynucleotide is RNA.

35. A chimeric gene comprising the isolated polynucleotide of Claim 31 operably linked to suitable regulatory sequences.

36. An isolated host cell comprising the chimeric gene of Claim 35.

37. An isolated host cell comprising an isolated polynucleotide of Claim 31.

38. The isolated host cell of Claim 37 wherein the isolated host is selected from the group consisting of yeast, bacteria, plant, and virus.

39. A virus comprising the isolated polynucleotide of Claim 31.

40. A polypeptide of at least 243 amino acids that has at least 90% identity based on the Clustal method of alignment when compared to a polypeptide selected from the group consisting of histidyl-tRNA synthetase polypeptides of SEQ ID NOs:18, 20 and 22.

41. A method of selecting an isolated polynucleotide that affects the level of expression of a aminoacyl-tRNA synthetase polypeptide in a plant cell, the method comprising the steps of:

(a) constructing an isolated polynucleotide comprising a nucleotide sequence of at least one of 30 contiguous nucleotides derived from a nucleotide sequence selected from the group consisting of SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37 and the complement of such nucleotide sequences;

(b) introducing the isolated polynucleotide into a plant cell; and

(c) measuring the level of an aminoacyl t-RNA synthetase polypeptide in the plant cell containing the polynucleotide.

42. The method of Claim 41 wherein the isolated polynucleotide consists of a nucleotide sequence selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35 and 37 that codes for the polypeptide selected from the group consisting of SEQ ID NOs:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36 and 38.

43. A method of selecting an isolated polynucleotide that affects the level of expression of a aminoacyl-tRNA synthetase polypeptide in a plant cell, the method comprising the steps of:

(a) constructing an isolated polynucleotide of any of Claims 1, 11, 21 or 31;

(b) introducing the isolated polynucleotide into a plant cell;

(c) measuring the level of an aminoacyl-tRNA synthetase polypeptide in the plant cell containing the polynucleotide; and

(d) comparing the level of aminoacyl-tRNA synthetase polypeptide in the plant cell containing the isolated polynucleotide with the level of aminoacyl-tRNA synthetase polypeptide in a plant cell that does not contain the polynucleotide.

44. A method of obtaining a nucleic acid fragment encoding a aminoacyl-tRNA synthetase polypeptide comprising the steps of:

(a) synthesizing an oligonucleotide primer comprising a nucleotide sequence of at least one of 30 contiguous nucleotides derived from a nucleotide sequence selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37 and the complement of such nucleotide sequences; and

(b) amplifying a nucleic acid sequence using the oligonucleotide primer.

45. A method of obtaining a nucleic acid fragment encoding the amino acid sequence encoding a aminoacyl-tRNA synthetase polypeptide comprising the steps of:

(a) probing a cDNA or genomic library with an isolated polynucleotide comprising a nucleotide sequence of at least one of 30 contiguous nucleotides derived from a nucleotide sequence selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37 and the complement of such nucleotide sequences;

(b) identifying a DNA clone that hybridizes with the isolated polynucleotide;

(c) isolating the identified DNA clone; and

(d) sequencing the cDNA or genomic fragment that comprises the isolated DNA clone.

46. A method for evaluating at least one compound for its ability to inhibit the activity of a aminoacyl-tRNA synthetase, the method comprising the steps of:

(a) transforming a host cell with a chimeric gene comprising a nucleic acid fragment encoding a aminoacyl-tRNA synthetase, operably linked to suitable regulatory sequences;

(b) growing the transformed host cell under conditions that are suitable for expression of the chimeric gene wherein expression of the chimeric gene results in production of the aminoacyl-tRNA synthetase encoded by the operably linked nucleic acid fragment in the transformed host cell;

(c) optionally purifying the aminoacyl-tRNA synthetase expressed by the transformed host cell;

(d) treating the aminoacyl-tRNA synthetase with a compound to be tested; and

(e) determining the activity of the aminoacyl-tRNA synthetase that has been treated with a test compound.

47. A composition comprising an isolated polynucleotide of Claim 1.

48. A composition comprising an isolated polynucleotide of Claim 11.

49. A composition comprising an isolated polynucleotide of Claim 21.

50. A composition comprising an isolated polynucleotide of Claim 31.

51. A composition comprising a polypeptide of Claim 10, Claim 20, Claim 30, or Claim 40.

52. An isolated polynucleotide comprising the nucleotide sequence comprising at least one of 30 contiguous nucleotides of nucleic acid sequences selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37 and the complement of such sequences.

53. An expression cassette comprising an isolated polynucleotide of Claim 1, Claim 11, Claim 21, or Claim 31 operably linked to a promoter.

54. A method for positive selection of a transformed cell comprising:

(a) transforming a plant cell with an expression cassette of Claim 53; and

(b) growing the transformed plant cell under conditions allowing expression of the polynucleotide in an amount sufficient to complement an amino-acyl t-RNA synthesis auxotroph in a plant cell to provide a positive selection means.

55. A method for positive selection of a transformed cell comprising:

(a) transforming a plant cell with a chimeric gene of Claim 5, Claim 15, Claim 25 or Claim 35; and

(b) growing the transformed plant cell under conditions allowing expression of the polynucleotide in an amount sufficient to complement an amino-acyl t-RNA synthesis auxotroph in a plant cell to provide a positive selection means.

56. The method of Claim 54 wherein the plant cell is a monocot.

57. The method of Claim 56 wherein the plant cell is a dicot.

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(54) Title: PLANT AMINOACYL–tRNA SYNTHETASES

(57) Abstract

This invention relates to an isolated nucleic acid fragment encoding an aminoacyl–tRNA synthetase. The invention also relates to the construction of a chimeric gene encoding all or a portion of the aminoacyl–tRNA synthetase, in sense or antisense orientation, wherein expression of the chimeric gene results in production of altered levels of the aminoacyl–tRNA synthetase in a transformed host cell.

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BB1270PCT

DECLARATION and POWER OF ATTORNEY

As a below-named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

PLANT AMINOACYL-tRNA SYNTHETASES

the specification of which is attached hereto unless the following box is checked:

■ was filed on **09 NOVEMBER 1999** as U.S. Application No. _____ or PCT International Application No. **PCT/US99/26478** and was amended on _____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is known to me to be material to patentability as defined in 37 CFR § 1.56.

I hereby claim foreign priority benefits under 35 U.S.C. § 119(a)-(d) or § 365(b) of any foreign application(s) for patent or inventor's certificate, or § 365(a) of any PCT International application which designated at least one country other than the United States, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or PCT International application having a filing date before that of the application on which priority is claimed.

Application No.	Country	Filing Date	Priority Claimed (Yes/No)

I hereby claim the benefit under 35 U.S.C. § 119(e) of any United States Provisional Application(s) listed below.

U.S. Provisional Application No.	U.S. Filing Date
60/107,789	10 NOVEMBER 1998

I hereby claim the benefit under 35 U.S.C. § 120 of any United States application(s), or § 365(c) of any PCT International Application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application or PCT International Application in the manner provided by the first paragraph of 35 U.S.C. § 112, I acknowledge the duty to disclose information which is known to me to be material to patentability as defined in 37 CFR § 1.56 which became available between the filing date of the prior application and the national or PCT International filing date of this application.

Application No.	Filing Date	Status (patented, pending or abandoned)

POWER OF ATTORNEY: I hereby appoint the following attorney(s) and/or agent(s) the power to prosecute this application and transact all business in the Patent and Trademark Office connected therewith:

Name: GREGORY J. FEULNER	Registration No.: 41,744

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

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GENERAL POWER OF ATTORNEY

(Concerning Several International Patent Applications)

The undersigned, Vernon R. Rice, Vice President and Assistant General Counsel of E. I. DU PONT DE NEMOURS AND COMPANY, 1007 Market Street, Wilmington, Delaware 19898 USA ("DuPont"), hereby confirms that the power to sign for DuPont has been granted to various individuals (as set forth in the attached excerpt from DuPont's Patent Board Rules of Procedure (January 1988), Appendix Section III.A.4), including the Chairman, Vice-Chairman, and those individuals who are Assistant Secretaries of the Patent Board. Currently these Assistant Secretaries are:

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Linda J. Davis	Dorothy W. Shafer
John E. Griffiths	Deborah A. Megimniss

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Birch, Linda D.	38,719	Kuller, Mark D.	31,925
Bowen, Jr., Alanson G.	24,027	Krukiel, Charles E.	27,344
Christenbury, Lynne M.	30,971	Jarnholm, Arne R.	30,396
Cotreau, William J.	36,490	Langworthy, John A.	32,255
Deitch, Gerald E.	30,457	Lerman, Bart E.	31,897
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Duffy, Roseanne R.	33,869	Mayer, Nancy S.	29,190
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Gorman, Thomas W.	31,959	Siegell, Barbara C.	30,684
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Hamby, William H.	31,521	Strickland, Frederick D.	39,041
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Jones, Brian C.	37,857	Walker, P. Michael	32,602
Joung, J. Kenneth	41,881	Wang, Chen	38,650

The undersigned ratifies fully all actions already taken by the above-named individuals in accordance with the authority granted hereby.

E. I. DU PONT DE NEMOURS AND COMPANY

By: _____

Vernon R. Rice
Vice President and Assistant General Counsel

Date: 5/1/2000

SEQUENCE LISTING

<110> E. I. du Pont de Nemours and Company

<120> Plant Aminoacyl-tRNA Synthetases

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Gly Asn Gly Gln Ile Ala Asp Trp Thr Asp Asp Glu Leu Asp Arg Thr
            420                 425                 430

Ser Glu Ala Ile Gly Tyr Gly Ala Val Lys Tyr Ser Asp Leu Lys Asn
        435                 440                 445

Asn Arg Leu Thr Asp Tyr Thr Phe Ser Phe Asp Gln Met Leu Ser Asp
    450                 455                 460

Lys Gly Asn Thr Ala Val Tyr Leu Gln Tyr Ala His Ala Arg Ile Cys
465                 470                 475                 480

Ser Ile Ile Arg Lys Ala Ser Lys Asp Val Glu Lys Leu Lys Met Thr
                485                 490                 495

Gly Ala Ile Thr Leu Gly His Pro Tyr Glu Arg Phe Leu Gly Leu His
            500                 505                 510

Leu Ile Gln Phe Thr Glu Val Val Glu Gln Ala Cys Ala Asp Leu Gln
        515                 520                 525

Pro His Arg Leu Cys Asp Tyr Leu Tyr Ser Leu Ser Leu Thr Phe Ser
    530                 535                 540

Lys Phe Tyr Thr Asn Cys Gln Val Val Gly Ser Pro Glu Glu Thr Ser
545                 550                 555                 560

Arg Leu Leu Leu Cys Glu Ala Thr Gly Ile Ile Met Arg Gln Cys Phe
                565                 570                 575

His Leu Leu Gly Ile Thr Pro Val His Lys Leu
            580                 585
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<210> 5
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<212> DNA
<213> Glycine max

<400> 5

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gattgtatat gttacagata ttgggcagca acagcacttt gatatgctat ttaaggccta  180
taggcgtgca ggttggttac caaaggatga gaatgcgtat ccaaaatgta ctcatatagg  240
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gatctgttcc attatcagga aatctggtaa agacatagaa gaagtaaaga gaaatgggaa  600
aatagtgttg gatcatgaag atgaacgtgc attggggctt catttgctac aatttcctga  660
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attcttcatc agatttttt gggatataca agtataggaa acttcacaat gaaaattgtt  960
caggcaaatt cgaccactcc ttccccctctt ccattttgtt aattttattt gagttgtaac 1020
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<210> 6
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<212> PRT
<213> Glycine max

<400> 6

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Val Glu Gly Val Asp Ile Pro Leu Ile Ala Val Lys Arg Asp Gly Gly
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Tyr Asn Tyr Phe Thr Thr Asp Leu Ala Ser Leu Trp Tyr Arg Leu Asn
            20                  25                  30

Glu Glu Lys Leu Glu Trp Ile Val Tyr Val Thr Asp Ile Gly Gln Gln
        35                  40                  45

Gln His Phe Asp Met Leu Phe Lys Ala Tyr Arg Arg Ala Gly Trp Leu
    50                  55                  60

Pro Lys Asp Glu Asn Ala Tyr Pro Lys Cys Thr His Ile Gly Phe Gly
65                  70                  75                  80

Leu Val Leu Gly Glu Asp Gly Lys Arg Phe Arg Thr Arg Ser Ser Glu
                85                  90                  95

Val Val Arg Leu Val Asp Leu Leu Asp Glu Ala Lys Arg Arg Cys Lys
            100                 105                 110

Ile Ala Ile Leu Glu Arg Asp Thr Thr Lys Asp Trp Ser Glu Glu Glu
        115                 120                 125

Ile Glu Lys Thr Ser Glu Ala Val Gly Tyr Gly Ala Val Lys Tyr Ala
    130                 135                 140

Asp Leu Lys Ile Asn Arg Leu Thr Asn Tyr Thr Phe Asn Phe Asp Gln
145                 150                 155                 160

Met Leu Asn Asp Lys Gly Asn Thr Ala Val Tyr Leu Leu Tyr Ala His
                165                 170                 175

Ala Arg Ile Cys Ser Ile Ile Arg Lys Ser Gly Lys Asp Ile Glu Glu
            180                 185                 190

Val Lys Arg Asn Gly Lys Ile Val Leu Asp His Glu Asp Glu Arg Ala
        195                 200                 205

Leu Gly Leu His Leu Leu Gln Phe Pro Glu Val Phe Glu Glu Ala Cys
    210                 215                 220

Thr Asn Leu Leu Pro Asn Phe Leu Cys Glu Tyr Leu Tyr Asn Leu Ala
225                 230                 235                 240

Glu Ile Phe Thr Lys Lys Phe Tyr Ala Asn Cys Gln Val Val Gly Ser
                245                 250                 255

Pro Glu Glu Thr Ser Arg Leu Leu Leu Cys Glu Ala Thr Val Thr Val
            260                 265                 270

Met Arg His Cys Phe Tyr Leu Leu Gly Ile Glu His Val Tyr Arg Leu
        275                 280                 285
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<211> 1041
<212> DNA
<213> Triticum aestivum

<400> 7
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cgaagtaaat cagaacttct ccagcgtctc actgaaaatg gtaaaattgt tgactggact 240
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gttggcacca ctaacatact ctgagtcaga ttgccccatt tgagaactga agcatctggt 960
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tcaaaaaaaa aaaaaaaaaa a 1041

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<211> 240
<212> PRT
<213> Triticum aestivum

<400> 8
Val Phe Phe Lys Ala Ala Arg Met Ala Gly Trp Leu Pro Asp Pro Lys
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Glu Lys Lys Phe Pro Lys Thr Ser His Val Gly Phe Gly Leu Val Leu
20 25 30

Gly Ala Asp Gly Lys Arg Phe Arg Thr Arg Ser Thr Glu Val Val Arg
35 40 45

Leu Gly Asp Leu Leu Asp Glu Ala Lys Ser Arg Ser Lys Ser Glu Leu
50 55 60

Leu Gln Arg Leu Thr Glu Asn Gly Lys Ile Val Asp Trp Thr Asp Glu
65 70 75 80

Glu Leu Glu Gln Thr Ser Lys Ala Val Gly Tyr Gly Ala Val Lys Tyr
85 90 95

Ala Asp Leu Lys Asn Asn Arg Leu Thr Asn Tyr Thr Phe Ser Phe Asp
100 105 110

Gln Met Leu Ser Asp Lys Gly Asn Thr Ala Val Tyr Leu Gln Tyr Ala
115 120 125

His Ala Arg Ile Cys Ser Ile Ile Arg Lys Ser Asn Met Asp Val Glu
130 135 140

Glu Leu Lys Val Ser Gly Asn Ile Ser Leu Ala His Pro Asp Glu Arg
145 150 155 160

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Val Leu Gly Leu Tyr Leu Ile Arg Tyr Ala Glu Ile Val Glu Glu Ala
165 170 175

Cys Thr Asn Leu Leu Pro Ser Val Leu Cys Glu Tyr Leu Tyr Asn Leu
180 185 190

Ser Glu Met Phe Thr Arg Phe Tyr Thr Asn Cys Gln Val Val Gly Ser
195 200 205

Pro Glu Glu Pro Ser Arg Leu Leu Leu Cys Glu Ala Thr Gly Val Val
210 215 220

Met Arg Gln Cys Phe Gln Leu Leu Gly Ile Thr Pro Val Tyr Met Leu
225 230 235 240

<210> 9
<211> 2572
<212> DNA
<213> Zea mays

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cgctgctgcc ctacgccgtc ttcgggctct tcgttgtcga cgagcgccac aaggttccta 180
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tattgttgca ttgtacactt taatattgta ctgtgattag gctttactcg cggtggatgt 2460
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<210> 10
<211> 715
<212> PRT
<213> Zea mays

<400> 10

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Ile Cys Ala Ala Lys Leu Val Gly Leu Pro Leu Thr Ile Asn His Ser
20 25 30

Leu Ala Ala Gly Ser Ala Pro Thr Leu Gln Phe Ala Ser Gly Glu Ser
35 40 45

Leu His Gly Val Asn Pro Ile Ile Leu Tyr Ile Ala Arg Gly Ala Ser
50 55 60

Ile Ala Ser Leu Ser Gly Lys Asn Asp Ile Glu Phe Gly His Val Val
65 70 75 80

Glu Trp Leu Glu Tyr Ala Pro Thr Phe Leu Ser Gly Ser Glu Phe Glu
85 90 95

Asn Ala Cys Leu Phe Val Asp Gly Phe Leu Ala Ser Arg Thr Phe Leu
100 105 110

Val Gly His Gly Leu Thr Ile Ala Asp Ile Ala Val Trp Ser Asn Leu
115 120 125

Ala Gly Ile Gly Gln Arg Trp Glu Ser Leu Arg Lys Ser Lys Lys Tyr
130 135 140

Gln Asn Leu Val Arg Trp Phe Asn Ser Ile Asp Ser Glu Tyr Lys Glu
145 150 155 160

Ala Leu Asn Glu Val Val Ala Ala Phe Val Gly Lys Arg Gly Ile Gly
165 170 175

Lys Ser Pro Ala Pro Ser Leu Lys Glu Lys Val His Asp Ser Lys Asp
180 185 190

Pro Ser Ala Pro Glu Val Asp Leu Pro Gly Ala Lys Val Gly Lys Val
195 200 205

Cys Val Arg Phe Ala Pro Glu Pro Ser Gly Tyr Leu His Ile Gly His
210 215 220

Ala Lys Ala Ala Leu Leu Asn Lys Tyr Phe Ala Glu Arg Tyr Gln Gly
225 230 235 240

Arg Leu Ile Val Arg Phe Asp Asp Thr Asn Pro Ser Lys Glu Ser Asn
245 250 255

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Glu Phe Val Glu Asn Leu Leu Lys Asp Ile Glu Thr Leu Gly Ile Lys
            260                 265                 270

Tyr Asp Ala Val Thr Tyr Thr Ser Asp Tyr Phe Pro Lys Leu Met Glu
        275                 280                 285

Met Ala Glu Ser Leu Ile Lys Gln Gly Lys Ala Tyr Ile Asp Asp Thr
    290                 295                 300

Pro Lys Glu Gln Met Arg Lys Glu Arg Met Asp Gly Ile Glu Ser Arg
305                 310                 315                 320

Cys Arg Asn Asn Thr Val Glu Glu Asn Leu Ser Leu Trp Lys Glu Met
                325                 330                 335

Val Asn Gly Thr Glu Arg Gly Met Gln Cys Cys Val Arg Gly Lys Leu
            340                 345                 350

Asp Met Gln Asp Pro Asn Lys Ser Leu Arg Asp Pro Val Tyr Tyr Arg
        355                 360                 365

Cys Asn Thr Asp Pro His His Arg Val Gly Ser Lys Tyr Lys Val Tyr
    370                 375                 380

Pro Thr Tyr Asp Phe Ala Cys Pro Phe Val Asp Ala Leu Glu Gly Val
385                 390                 395                 400

Thr His Ala Leu Arg Ser Ser Glu Tyr His Asp Arg Asn Ala Gln Tyr
                405                 410                 415

Tyr Arg Ile Leu Gln Asp Met Gly Leu Arg Arg Val Glu Ile Tyr Glu
            420                 425                 430

Phe Ser Arg Leu Asn Met Val Tyr Thr Leu Leu Ser Lys Arg Lys Leu
        435                 440                 445

Leu Trp Phe Val Gln Asn Lys Lys Val Glu Asp Trp Thr Asp Pro Arg
    450                 455                 460

Phe Pro Thr Val Gln Gly Ile Val Arg Arg Gly Leu Lys Val Glu Ala
465                 470                 475                 480

Leu Ile Gln Phe Ile Leu Gln Gln Gly Ala Ser Lys Asn Leu Asn Leu
                485                 490                 495

Met Glu Trp Asp Lys Leu Trp Thr Ile Asn Lys Lys Ile Ile Asp Pro
            500                 505                 510

Val Cys Ala Arg His Thr Ala Val Leu Lys Asp Gln Arg Val Ile Phe
        515                 520                 525

Thr Leu Thr Asn Gly Pro Glu Glu Pro Phe Val Arg Ile Leu Pro Arg
    530                 535                 540

His Lys Lys Phe Glu Gly Ala Gly Lys Lys Ala Thr Thr Phe Ala Asn
545                 550                 555                 560

Arg Ile Trp Leu Asp Tyr Ala Asp Ala Ala Ala Ile Asn Lys Gly Glu
                565                 570                 575
```

```
Glu Val Thr Leu Met Asp Trp Gly Asn Ala Ile Val Lys Glu Ile Lys
            580                 585                 590

Val Glu Ser Gly Val Ile Thr Glu Leu Val Gly Glu Leu His Leu Glu
        595                 600                 605

Gly Ser Val Lys Thr Thr Lys Leu Lys Ile Thr Trp Leu Ala Asp Ile
    610                 615                 620

Glu Glu Leu Val Pro Leu Ser Leu Val Glu Phe Asp Tyr Leu Ile Ser
625                 630                 635                 640

Lys Lys Lys Leu Glu Glu Asp Glu Asp Phe Leu Asp Asn Leu Asn Pro
                645                 650                 655

Cys Thr Arg Arg Glu Ile Pro Ala Leu Gly Asp Ala Asn Met Arg Asn
            660                 665                 670

Ile Lys Arg Gly Glu Ile Ile Gln Leu Glu Arg Lys Gly Tyr Tyr Arg
        675                 680                 685

Cys Asp Ala Pro Phe Ile Arg Ser Ser Lys Pro Val Val Leu Phe Ala
    690                 695                 700

Ile Pro Asp Gly Arg Gln Gln Ala Ser Leu Ser
705                 710                 715
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<210> 11
<211> 1920
<212> DNA
<213> Oryza sativa

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<222> (139)

<220>
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<222> (238)

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<221> unsure
<222> (431)

<400> 11

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tccgcctccg cctcggcgnc accggacggc gccggcggcc cggtgcgggc ccgttcgcgc  180
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cgcgcggtcc aaggggggca agttcgtgct ccgcatcgag gacaccgact tcgagaggtc  300
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<210> 12
<211> 555
<212> PRT
<213> Oryza sativa

<220>
<221> UNSURE
<222> (130)

<400> 12

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His Leu Arg Arg Pro Ser Pro Ser Ala Pro Pro Pro Pro Pro Arg Arg
             20                  25                  30

His Arg Thr Ala Pro Ala Ala Arg Cys Gly Pro Val Arg Ala Val Ala
         35                  40                  45

Asp Gly Asn Leu His Val Gly Gly Ala Arg Thr Ala His Phe Asn Tyr
     50                  55                  60

Leu Phe Ala Arg Ser Lys Gly Gly Lys Phe Val Leu Arg Ile Glu Asp
 65                  70                  75                  80

Thr Asp Phe Glu Arg Ser Thr Lys Lys Ser Glu Glu Ala Val Leu Ser
                 85                  90                  95

Asp Leu Ala Trp Leu Gly Leu Asp Trp Asp Glu Gly Pro Asp Val Gly
            100                 105                 110

Gly Glu Tyr Gly Pro Asp Arg Gln Ser Glu Arg Asn Ser Met Tyr Lys
        115                 120                 125

Gln Xaa Ala Glu Lys Leu Met Glu Ser Gly Ala Val Tyr Gln Cys Phe
    130                 135                 140

Tyr Ser Ser Glu Gly Leu Glu Gln Met Lys Glu Thr Ala Ser Arg Cys
145                 150                 155                 160

Asn Leu Pro Pro Val Tyr Ile Gly Lys Trp Gly Thr Ala Ser Asp Ala
                165                 170                 175
```

Glu Ile Gln Gln Glu Leu Glu Lys Gly Thr Pro Tyr Thr Tyr Arg Phe
180 185 190

Arg Val Pro Lys Glu Gly Ser Leu Lys Ile Asn Asp Leu Ile Arg Gly
195 200 205

Glu Val Ser Trp Asn Leu Asp Thr Leu Gly Asp Phe Val Ile Met Arg
210 215 220

Ser Asn Gly Gln Pro Val Tyr Asn Phe Cys Val Thr Val Asp Asp Ala
225 230 235 240

Thr Met Arg Ile Ser His Val Ile Arg Ala Glu Glu His Leu Pro Asn
245 250 255

Thr Leu Arg Gln Ala Leu Ile Tyr Lys Ala Leu Gly Phe Pro Met Pro
260 265 270

Ser Phe Ala His Val Ser Leu Ile Leu Ala Pro Asp Arg Ser Lys Leu
275 280 285

Ser Lys Arg His Gly Ala Thr Ser Val Gly Gln Tyr Lys Glu Met Gly
290 295 300

Tyr Leu Pro Gln Ala Met Val Asn Tyr Leu Ala Leu Leu Gly Trp Gly
305 310 315 320

Asp Gly Thr Glu Asn Glu Phe Phe Thr Ile Asp Asp Leu Val Glu Lys
325 330 335

Phe Thr Ile Asn Arg Val Asn Lys Ser Gly Ala Val Phe Asp Ala Val
340 345 350

Lys Leu Lys Trp Met Asn Gly Gln His Leu Arg Ser Phe Pro Pro Asp
355 360 365

Val Leu Ile Lys Ser Phe Glu Asp Arg Trp Lys Asp Thr Gly Ile Leu
370 375 380

Gln Glu Ser Glu Ser Gly Phe Ala Lys Glu Ala Ala Glu Leu Leu Lys
385 390 395 400

Asp Gly Ile Asp Leu Ile Thr Asp Ala Asp Ala Ala Leu Ser Asn Leu
405 410 415

Leu Ser Tyr Pro Leu His Ala Thr Leu Ser Ser Asp Glu Ala Lys Ser
420 425 430

Val Val Gln Asp Lys Leu Ser Glu Val Ala Ser Gly Leu Ile Ser Ala
435 440 445

Tyr Asp Ser Gly Glu Leu Cys Gln Ala Leu Ala Glu Gly Arg Asp Gly
450 455 460

Trp Gln Lys Trp Val Lys Ile Phe Gly Lys Ser Leu Lys Arg Lys Gly
465 470 475 480

Lys Ser Leu Phe Met Pro Leu Arg Val Leu Leu Thr Gly Lys Leu His
485 490 495

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Gly Pro Asp Met Gly Gly Thr Val Val Leu Ile His Lys Ala Gly Thr
            500                 505                 510

Cys Gly Ala Val Thr Gln Gln Ser Gly Phe Val Asn Leu Asp Glu Arg
        515                 520                 525

Phe Arg Ile Leu Lys Glu Val Glu Trp Glu Ser Leu Val Gln Glu Gln
    530                 535                 540

Glu Ser Pro Ala Glu Thr Ala Val Pro Ala Ser
545                 550                 555
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<210> 13
<211> 731
<212> DNA
<213> Glycine max

<400> 13

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caggtaaatg ggccagtgca acaaatgagg aagtagaaga agagctagca aaaggaactc 600
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agcctgttta t                                                     731
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<210> 14
<211> 404
<212> PRT
<213> Glycine max

<400> 14

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Gly Ala Arg Thr Ala Leu Phe Asn Tyr Leu Phe Ala Arg Ser Lys Gly
             20                  25                  30

Gly Lys Phe Val Leu Arg Ile Glu Asp Thr Asp Leu Glu Arg Ser Thr
         35                  40                  45

Arg Glu Ser Glu Glu Ala Met Leu Lys Asp Leu Ser Trp Leu Gly Leu
     50                  55                  60

Asp Trp Asp Glu Gly Pro Gly Val Gly Gly Asp Tyr Gly Pro Tyr Arg
 65                  70                  75                  80

Gln Ser Asp Arg Asn Ser Leu Tyr Lys Gln Phe Ala Asp Asn Leu His
                 85                  90                  95

Gln Ser Gly His Val Tyr Arg Cys Phe Cys Ser Asn Glu Glu Leu Glu
            100                 105                 110
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09831683 .051001


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Lys Met Lys Glu Asp Ala Lys Leu Lys Gln Leu Pro Pro Val Tyr Thr
        115                 120                 125

Gly Lys Trp Ala Ser Ala Thr Asn Glu Glu Val Glu Glu Glu Leu Ala
    130                 135                 140

Lys Gly Thr Pro Tyr Thr Tyr Arg Phe Arg Val Pro Lys Gly Ser Leu
145                 150                 155                 160

Lys Ile Asn Asp Gln Ile Arg Gly Glu Val Ser Trp Asn Leu Asp Thr
                165                 170                 175

Leu Gly Asp Phe Val Ile Met Arg Ser Asn Gly Gln Pro Val Tyr Asn
            180                 185                 190

Phe Cys Val Thr Val Asp Asp Ala Thr Met Ala Ile Ser His Val Ile
        195                 200                 205

Arg Ala Glu Glu His Leu Pro Asn Thr Leu Arg Gln Ala Leu Ile Tyr
    210                 215                 220

Lys Ala Leu Gly Phe Pro Met Pro His Phe Ala His Val Ser Leu Ile
225                 230                 235                 240

Leu Ala Pro Asp Arg Ser Lys Leu Ser Lys Arg His Gly Ala Thr Ser
                245                 250                 255

Val Gly Gln Phe Arg Asp Met Gly Tyr Leu Pro Gln Ala Met Val Asn
            260                 265                 270

Tyr Leu Ala Leu Leu Gly Trp Gly Asp Gly Thr Glu Asn Glu Phe Phe
        275                 280                 285

Thr Leu Glu Gln Leu Val Glu Lys Phe Thr Ile Glu Arg Val Asn Lys
    290                 295                 300

Ser Gly Ala Ile Phe Asp Ser Thr Lys Leu Arg Trp Met Asn Gly Gln
305                 310                 315                 320

His Leu Arg Ser Leu Pro Ser Glu Glu Leu Asn Arg Ile Ile Gly Glu
                325                 330                 335

Arg Trp Lys Asp Ala Gly Ile Ala Thr Glu Ser Gln Gly Ile Phe Ile
            340                 345                 350

Gln Asp Ala Val Leu Leu Leu Lys Asp Gly Ile Asp Leu Ile Thr Asp
        355                 360                 365

Ser Glu Lys Ala Leu Ser Ser Leu Leu Ser Tyr Pro Leu Tyr Glu Thr
    370                 375                 380

Leu Ala Ser Ala Glu Gly Lys Pro Ile Leu Glu Asp Gly Val Ser Glu
385                 390                 395                 400

Val Ala Lys Ser
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<210> 15
<211> 407

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<212> DNA
<213> Triticum aestivum

<220>
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gccctcttca attacctgat tgcaaaagct acacgcggta aattcatcct acgcatagag 240
gacacagatn agtcaaggac tgttcctggt gcgattgaaa aactctgcgc tgntttgaga 300
tgggggggtt taaaaaggga taaaagggct ggtccccaat ngaccgcaan ngggcctttc 360
aaaaatctca aaagactttt aangttataa aaaaaaacnc nccataa 407

<210> 16
<211> 79
<212> PRT
<213> Triticum aestivum

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<222> (55)

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1 5 10 15

09931682 .053001

Ala Pro Ser Pro Thr Gly Asn Leu His Leu Gly Ser Leu Arg Thr Ala
20 25 30

Leu Phe Asn Tyr Leu Ile Ala Lys Ala Thr Arg Gly Lys Phe Ile Leu
35 40 45

Arg Ile Glu Asp Thr Asp Xaa Ser Arg Thr Val Pro Gly Ala Ile Glu
50 55 60

Lys Leu Cys Ala Xaa Leu Arg Trp Gly Gly Leu Lys Arg Asp Lys
65 70 75

<210> 17
<211> 2387
<212> DNA
<213> Zea mays

<400> 17
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gaaggggatg cttgacaatg ttttgattga ttcagatgcc gtatcagtct tgaaggggct 420
gtacaaccac ttgctcaagt tcagggactt tcttgcctgg gaagcagctg tggccatggc 480
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<210> 18
<211> 495
<212> PRT
<213> Zea mays

<400> 18

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Asn Ser Ala Ser Val Ala Glu Trp Ala Thr Ser Leu Ser Leu Leu Phe
             20                  25                  30

Asp Pro Lys Cys Pro Gly Leu Glu Ser Leu Val Glu Lys Val Lys Glu
         35                  40                  45

Ile Val Glu Ser Asn Glu Val Arg Arg Leu Pro Lys Ile Pro Lys Gly
     50                  55                  60

Thr Arg Asp Phe Gly Lys Glu Gln Met Ala Ile Arg Glu Arg Ala Phe
 65                  70                  75                  80

Ser Ile Ile Thr Ser Val Phe Lys Met His Gly Ala Thr Ala Leu Asp
                 85                  90                  95

Thr Pro Val Phe Glu Leu Arg Glu Thr Leu Met Gly Lys Tyr Gly Glu
            100                 105                 110

Asp Ser Lys Leu Ile Tyr Asp Leu Ala Asp Gln Gly Gly Glu Leu Cys
        115                 120                 125

Ser Leu Arg Tyr Asp Leu Thr Val Pro Phe Ala Arg Tyr Val Ala Met
    130                 135                 140

Asn Ser Ile Ser Ala Leu Lys Arg Tyr Gln Ile Ala Lys Val Tyr Arg
145                 150                 155                 160

Arg Asp Asn Pro Ser Lys Gly Arg Tyr Arg Glu Phe Tyr Gln Cys Asp
                165                 170                 175

Phe Asp Ile Ala Gly Val Tyr Glu Pro Met Glu Pro Asp Phe Glu Val
            180                 185                 190

Ile Lys Val Leu Thr Glu Leu Leu Asn Gln Leu Asp Ile Gly Thr Tyr
        195                 200                 205

Glu Ile Lys Leu Asn His Arg Lys Leu Leu Asp Gly Met Leu Glu Ile
    210                 215                 220

Cys Gly Val Pro Pro Gln Lys Phe Arg Thr Val Cys Ser Ser Ile Asp
225                 230                 235                 240

Lys Leu Asp Lys Gln Thr Phe Glu Gln Val Lys Lys Glu Leu Val Asp
                245                 250                 255

Glu Lys Gly Ile Ser Asn Glu Thr Ala Asp Glu Ile Gly Asn Leu Val
            260                 265                 270

Lys Thr Arg Gly Pro Pro Leu Glu Val Leu Met Glu Leu Arg Lys Glu
        275                 280                 285
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Gly Ser Lys Phe Met Asn Asn Val Gly Ser Val Ala Ala Leu Asn Glu
    290                 295                 300

Leu Glu Ile Leu Phe Lys Ala Leu Asp Lys Ala Asn Ala Ile Ser Lys
305                 310                 315                 320

Ile Thr Phe Asp Leu Ser Leu Ala Arg Gly Leu Asp Tyr Tyr Thr Gly
                325                 330                 335

Val Ile Tyr Glu Ala Val Phe Lys Gly Ala Ala Gln Val Gly Ser Ile
            340                 345                 350

Ala Ala Gly Gly Arg Tyr Asp Asn Leu Val Gly Met Phe Ser Gly Lys
        355                 360                 365

Gln Ile Pro Ala Val Gly Val Ser Leu Gly Ile Glu Arg Val Phe Ala
    370                 375                 380

Ile Met Glu Gln Gln Glu Lys Glu Arg Asn Glu Lys Ile Arg Pro Thr
385                 390                 395                 400

Glu Thr Glu Val Leu Val Ser Ile Leu Gly Lys Asp Leu Thr Leu Ala
                405                 410                 415

Ala Glu Leu Val Ser Glu Leu Trp Asn Ala Gly Ile Lys Ala Glu Phe
            420                 425                 430

Lys Leu Thr Thr Arg Val Ala Asn His Ile Lys Tyr Ala Leu Gln Ser
        435                 440                 445

Ser Ile Pro Trp Met Val Leu Val Gly Glu Ser Glu Leu Gln Lys Gly
    450                 455                 460

Thr Val Lys Leu Lys Asp Val Glu Ala Asn Gln Glu Glu Glu Val Asp
465                 470                 475                 480

Arg Lys Asp Phe Val Arg Glu Leu Lys Lys Arg Leu Ser Lys Ser
                485                 490                 495
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<210> 19
<211> 754
<212> DNA
<213> Glycine max

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<220>
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<222> (740)

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gtcgaaaggt tctgcaagaa gtattaaatt gttattcagt accagaaaat ttatttggca 180
aggtctgcgt cattattgat aaaattgaga aaattccagc tgacgagata aagaaagagt 240
tgaaagctgt tggtctatct caagaggctg tccaggagct attgcaagtc ctttctgtga 300
agtcattgac cgagttagaa gagagacttg ggagcagtgg ggaagcagtt gctgatctga 360
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cagttgttcg aggtcttgct tactacactg gcattgtatt tgagggtttt gaccgagaag 480
gaaagctgcg agctatctgt ggtggtggtc gatatgatca tttgttctca acttttggtg 540
ctgatgacat tgctgcatgt ggttttggat ttggtgatgc aagtcatagt ggaattgctc 600
aaaagagaan ggtctgttac cgggaagctt aacttgcaaa tagatgacat tgtgtgtgcc 660
ttggaccaaa gatcttcaag ggatgtgctg ctatgggccc caacaatctc agngaaaaan 720
ggcaaattgt tgaagttggn tttgggaaaa caaa 754

<210> 20
<211> 243
<212> PRT
<213> Glycine max

<220>
<221> UNSURE
<222> (6)

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<222> (203)

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<221> UNSURE
<222> (235)

<220>
<221> UNSURE
<222> (238)

<400> 20
Asn Met Asp Ile Ile Xaa Val Pro Gly Val Met Ala Glu Ala Glu Leu
1 5 10 15

Ile Ala Ser Ile Val Thr Leu Phe Lys Arg Ile Gly Ile Thr Glu Ser
20 25 30

Asp Val Gly Phe Lys Val Ser Ser Arg Lys Val Leu Gln Glu Val Leu
35 40 45

Asn Cys Tyr Ser Val Pro Glu Asn Leu Phe Gly Lys Val Cys Val Ile
50 55 60

Ile Asp Lys Ile Glu Lys Ile Pro Ala Asp Glu Ile Lys Lys Glu Leu
65 70 75 80

Lys Ala Val Gly Leu Ser Gln Glu Ala Val Gln Glu Leu Leu Gln Val
85 90 95

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Leu Ser Val Lys Ser Leu Thr Glu Leu Glu Glu Arg Leu Gly Ser Ser
            100                 105                 110

Gly Glu Ala Val Ala Asp Leu Lys Gln Leu Phe Ser Leu Ala Glu Lys
        115                 120                 125

Ile Gly Tyr Ser Lys Trp Leu Gln Phe Asp Ala Ser Val Val Arg Gly
    130                 135                 140

Leu Ala Tyr Tyr Thr Gly Ile Val Phe Glu Gly Phe Asp Arg Glu Gly
145                 150                 155                 160

Lys Leu Arg Ala Ile Cys Gly Gly Gly Arg Tyr Asp His Leu Phe Ser
                165                 170                 175

Thr Phe Gly Ala Asp Asp Ile Ala Ala Cys Gly Phe Gly Phe Gly Asp
            180                 185                 190

Ala Ser His Ser Gly Ile Ala Gln Lys Arg Xaa Val Cys Tyr Arg Glu
        195                 200                 205

Ala Leu Ala Asn Arg His Cys Val Cys Leu Gly Pro Lys Ile Phe Lys
    210                 215                 220

Gly Cys Ala Ala Met Gly Pro Asn Asn Leu Xaa Glu Lys Xaa Gln Ile
225                 230                 235                 240

Val Glu Val
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<210> 21
<211> 1164
<212> DNA
<213> Triticum aestivum

<400> 21

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tgacaagctg gggaaactga gtagggaaga aattgagaag gaattgattt caactgggct  180
gtcatctgaa gcagtacagg gcatcattga agtgctctct ctcaagtcac tgtccaaact  240
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<210> 22
<211> 271

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<212> PRT
<213> Triticum aestivum

<400> 22
Lys Leu Gly Ile Thr Ser Ser Asp Val Gly Ile Arg Leu Ser Ser Arg
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Lys Val Leu Gln Ala Val Leu Asp Met Tyr Ser Val Pro Gln His Leu
20 25 30

Phe Thr Gln Val Cys Val Ile Val Asp Lys Leu Gly Lys Leu Ser Arg
35 40 45

Glu Glu Ile Glu Lys Glu Leu Ile Ser Thr Gly Leu Ser Ser Glu Ala
50 55 60

Val Gln Gly Ile Ile Glu Val Leu Ser Leu Lys Ser Leu Ser Lys Leu
65 70 75 80

Glu Glu Val Leu Gly Ser Gly Val Glu Ala Val Ala Asp Leu Lys Lys
85 90 95

Leu Phe Ser Leu Ala Glu Gln Tyr Gly Tyr Ser Asp Trp Ile Cys Phe
100 105 110

Asp Ala Ser Val Val Arg Gly Leu Ala Tyr Tyr Thr Gly Ile Val Phe
115 120 125

Glu Ala Phe Asp Arg Glu Gly Glu Leu Arg Ala Ile Cys Gly Gly Gly
130 135 140

Arg Tyr Asp Arg Leu Leu Ser Thr Phe Gly Thr Glu Asp Val Pro Ala
145 150 155 160

Cys Gly Phe Gly Phe Gly Asp Ala Val Ile Val Glu Leu Leu Lys Glu
165 170 175

Lys Gly Leu Leu Pro Asp Leu Pro Arg Gln Ile Asp Asp Ile Val Phe
180 185 190

Pro Leu Asp Glu Glu Leu Glu Gly Pro Ala Ser Ser Val Ala Ser Cys
195 200 205

Leu Arg Lys Lys Gly Arg Ser Val Asp Leu Val Glu Asp Lys Arg Leu
210 215 220

Lys Trp Val Phe Lys His Ala Glu Arg Ile Asn Ala Ser Arg Leu Ile
225 230 235 240

Leu Val Gly Lys Ser Glu Trp Glu Arg Gly Met Val Arg Val Lys Ile
245 250 255

Leu Ser Thr Arg Glu Glu Phe Glu Val Lys Ala Gly Glu Leu Gln
260 265 270

<210> 23
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<212> DNA
<213> Zea mays

<220>
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<400> 23
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tcaatgttga gcaggcagag tggatcatat atgttacaga tgttggtcag cagcagcact 240
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<210> 24
<211> 221
<212> PRT
<213> Zea mays

<220>
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<222> (96)

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<222> (156)

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Gly Leu Ile Lys Glu Ser Glu Gly Ala Arg Val Ile Phe Ile Gln Gly
20 25 30

His Gln Ile Pro Leu Ile Val Val Lys Ser Asp Gly Gly Phe Asn Tyr
35 40 45

Ala Ser Thr Asp Leu Thr Ala Leu Trp Tyr Arg Leu Asn Val Glu Gln
50 55 60

Ala Glu Trp Ile Ile Tyr Val Thr Asp Val Gly Gln Gln Gln His Phe
65 70 75 80

Asp Met Val Phe Ser Ala Ala Lys Met Ala Gly Trp Leu Pro Asp Xaa
85 90 95

Glu Lys Lys Phe Pro Lys Thr Ser His Val Gly Phe Gly Leu Phe Leu
100 105 110

Val Gln Asp Gly Lys Arg Phe Arg Thr Arg Ser Thr Glu Val Val Arg
115 120 125

Leu Val Glu Leu Leu Asp Glu Ala Lys Ser Arg Ser Lys Ser Glu Leu
130 135 140

Thr Glu Asn Gly Lys Ile Val Asp Trp Thr Asp Xaa Glu Leu Glu Gln
145 150 155 160

Thr Ser Glu Ala Val Gly Tyr Gly Ala Val Lys Tyr Ala Asp Leu Lys
165 170 175

Asn Asn Arg Leu Thr Asn Tyr Thr Phe Ser Phe Glu Gln Met Leu Ser
180 185 190

Asp Lys Gly Asn Thr Ala Val Tyr Leu Gln Tyr Ala His Ala Arg Ile
195 200 205

Cys Ser Ile Ile Arg Lys Ser Asn Lys Asn Val Xaa Asp
210 215 220

<210> 25
<211> 551
<212> DNA
<213> Oryza sp.

<220>
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<400> 25
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ggttttgggg attaccagtg caacaatgct atgagtgtat tttcaagaat aagaggatcc 180
gcaacaaact tccgtaaccc catggcagtt gggcaggcaa ttgcaaataa cctcccccag 240
tcaaatatta tcgaatccat ctctgttgcc gganctggtt acattaacat aacgttatcc 300
agcaattgga ttgcacagag gatacaaaga catgcttggt tgtgggaatc aaaacatggg 360
gaacaatcct taacctgttt aagaaggcaa ntgctggntt tttcaanccc caataattgc 420
aaaaagaana tgcaagttgg gcaataatna aggncaacaa taaatngggg natancccaa 480
ctcaaaangg ttgnggntca caaaanggtt aanttcntcn acgtaaacan gttgggaaac 540
nggggnacac a 551

<210> 26
<211> 68
<212> PRT
<213> Oryza sp.

<220>
<221> UNSURE
<222> (51)

<400> 26
Phe Gly Asp Tyr Gln Cys Asn Asn Ala Met Ser Val Phe Ser Arg Ile
1 5 10 15

Arg Gly Ser Ala Thr Asn Phe Arg Asn Pro Met Ala Val Gly Gln Ala
20 25 30

Ile Ala Asn Asn Leu Pro Gln Ser Asn Ile Ile Glu Ser Ile Ser Val
35 40 45

Ala Gly Xaa Gly Tyr Ile Asn Ile Thr Leu Ser Ser Asn Trp Ile Ala
50 55 60

Gln Arg Ile Gln
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<210> 27
<211> 411

<212> DNA
<213> Glycine max

<220>
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<400> 27
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acaactgatc tagcatcact ttggtatcgt ctaaatgaag aaaaacttga atggattgta 120
tatgttacag atattgggca gcaacagcac tttgatatnc tattnaaggc ctataggcgt 180
gcaggttggt taccaaagga tgagaatgcg tatccaaaat gtactcatat aggttttggt 240
cttgttcttg gggaagatgg aaaacgattt cggactcgca ncagtnangt tgttcgatta 300
gttgattact tgatgaagct aaaangcgct gtaaaattgc cntcttgaaa cgtgatacaa 360
ctaaaggatt ggnctgaagg aggagatcga gaaaacatcc gaagcagttg g 411

<210> 28
<211> 115
<212> PRT
<213> Glycine max

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<222> (6)

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<222> (94)

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<222> (96)

<220>
<221> UNSURE
<222> (109)

<400> 28

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Val Glu Gly Val Asp Xaa Pro Leu Ile Ala Val Xaa Arg Asp Gly Gly
  1               5                  10                  15

Xaa Asn Tyr Phe Thr Thr Asp Leu Ala Ser Leu Trp Tyr Arg Leu Asn
            20                  25                  30

Glu Glu Lys Leu Glu Trp Ile Val Tyr Val Thr Asp Ile Gly Gln Gln
        35                  40                  45

Gln His Phe Asp Xaa Leu Xaa Lys Ala Tyr Arg Arg Ala Gly Trp Leu
    50                  55                  60

Pro Lys Asp Glu Asn Ala Tyr Pro Lys Cys Thr His Ile Gly Phe Gly
65                  70                  75                  80

Leu Val Leu Gly Glu Asp Gly Lys Arg Phe Arg Thr Arg Xaa Ser Xaa
                85                  90                  95

Val Val Arg Leu Val Asp Tyr Leu Met Lys Leu Lys Xaa Ala Val Lys
            100                 105                 110

Leu Pro Ser
        115
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<210> 29
<211> 565

<212> DNA
<213> Triticum sp.

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<222> (350)

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<222> (548)

<220>
<221> unsure
<222> (555)

<400> 29
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caaaaacgag tcatgttggg tttggccttg ttcttggagc agatggcaag cgcttccgaa 120
ctcgtagtac tgaggttgtt cggttgggaa gacctacttg atgaggctaa atctcgaagt 180
aaatcaagaa cttctccaag cgtctcactg gaaaatggta aaattgttga ctggactgat 240
gaaggaacta agagcaaact tcaaaaggca gtaagatatg gcgctgtcaa agtatgcggg 300
tctgaaagaa taaccgactg actaattaca cttcaacttt gattcaagan ctaagtgaca 360
agggaaatac tgctgtcnac ttcaataagc caagcccgta cctcccanca ttcnaaaacc 420
caacatggtg tnnaaaacta aaangatggg anattccncc tgccanccaa atagctgcct 480
gggacgnact aacngtatgc aanatgttaa aaggatgaca acncttccaa tgtcngggng 540
aaactatnac taccnaagta aaagt 565

<210> 30
<211> 33
<212> PRT
<213> Triticum sp.

<400> 30
Glu Lys Lys Phe Pro Lys Thr Ser His Val Gly Phe Gly Leu Val Leu
1 5 10 15

Gly Ala Asp Gly Lys Arg Phe Arg Thr Arg Ser Thr Glu Val Val Arg
20 25 30

Leu

<210> 31
<211> 546
<212> DNA
<213> Oryza sp.

<220>
<221> unsure
<222> (448)

<220>
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<222> (512)

<400> 31
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catcgatttg atcactgatg ctgacgcagc cctttcaaac ctgttgtcgt atcccctcca 180

tgctacatta agcagtgatg aagctaaatc tgtggtgcaa gacaagcttt ctgaggttgc 240
atcaggactc atttctgctt atgatagcgg tgaactttgt caagcactag ctgagggccg 300
tgatggttgg cagaagtggg tgaaaatttt tggcaaatca cttaaaagaa agggaaagtc 360
actctttatg ccgctccgtg tactgctgac tggcaagctt catgggcctg acatgggcgg 420
caccgtagtc ctcatacaca aagccggnac tgtggagcgg tcactcaaca atccggtttc 480
gtaaatctcg acgagaggtc agaatcctga angagtggag tggagtcact ggtacaggac 540
aagatc 546

<210> 32
<211> 147
<212> PRT
<213> Oryza sp.

<400> 32

Pro	Pro	Asp	Val	Leu	Ile	Lys	Ser	Phe	Glu	Asp	Arg	Trp	Lys	Asp	Thr
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Gly	Ile	Leu	Gln	Glu	Ser	Glu	Ser	Gly	Phe	Ala	Lys	Glu	Ala	Ala	Glu
			20					25					30		
Leu	Leu	Lys	Asp	Gly	Ile	Asp	Leu	Ile	Thr	Asp	Ala	Asp	Ala	Ala	Leu
		35					40					45			
Ser	Asn	Leu	Leu	Ser	Tyr	Pro	Leu	His	Ala	Thr	Leu	Ser	Ser	Asp	Glu
	50					55					60				
Ala	Lys	Ser	Val	Val	Gln	Asp	Lys	Leu	Ser	Glu	Val	Ala	Ser	Gly	Leu
65					70					75					80
Ile	Ser	Ala	Tyr	Asp	Ser	Gly	Glu	Leu	Cys	Gln	Ala	Leu	Ala	Glu	Gly
				85					90					95	
Arg	Asp	Gly	Trp	Gln	Lys	Trp	Val	Lys	Ile	Phe	Gly	Lys	Ser	Leu	Lys
			100					105					110		
Arg	Lys	Gly	Lys	Ser	Leu	Phe	Met	Pro	Leu	Arg	Val	Leu	Leu	Thr	Gly
		115					120					125			
Lys	Leu	His	Gly	Pro	Asp	Met	Gly	Gly	Thr	Val	Val	Leu	Ile	His	Lys
	130					135					140				
Ala	Gly	Thr													
145															

<210> 33
<211> 524
<212> DNA
<213> Glycine max

<220>
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<222> (386)

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<400> 33
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tctccgaaca accaccaccc gttcgcgttc gtttcgctcc ttctcccacc ggaaacctcc 180
acgtcggcgg tgcccgaacg gccctcttca actacttgtt cgcaaggtcc aaaggtggga 240
aatttgtgct gagaattgag gacactgact tggagaggtc caagtaggga gtctgaggag 300
gccatgctca aagatctttc ttggcttgga cttgattggg atgaagggcc tgggtgttgg 360
aggggattat ggtccttaaa aggcantctg agaaggaatt ccttatacaa acaatatgcc 420
ggngaaacta cacaaatccg ggcaagttta accgctgcnt tctggtccaa agagggaact 480
nanagnaaat gaaaggaggt tgctaaacta aagcaactgg cccc 524

<210> 34
<211> 94
<212> PRT
<213> Glycine max

<220>
<221> UNSURE
<222> (63)

<400> 34
Gln Arg Arg Arg Phe Ser Val Ser Ala Leu Ser Glu Gln Pro Pro Pro
1 5 10 15

Val Arg Val Arg Phe Ala Pro Ser Pro Thr Gly Asn Leu His Val Gly
20 25 30

Gly Ala Arg Thr Ala Leu Phe Asn Tyr Leu Phe Ala Arg Ser Lys Gly
35 40 45

Gly Lys Phe Val Leu Arg Ile Glu Asp Thr Asp Leu Glu Arg Xaa Ser
50 55 60

Arg Glu Ser Glu Glu Ala Met Leu Lys Asp Leu Ser Trp Leu Gly Leu
65 70 75 80

Asp Trp Asp Lys Gly Leu Gly Val Gly Gly Asp Tyr Gly Pro
85 90

<210> 35
<211> 506
<212> DNA
<213> Glycine max

<220>
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<400> 35
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gtcgaaaggt tctgcaagaa gtattaaatt gttattcagt accagaaaat ttatttggca 180
aggtctgcgt cattattgat aaaattgaga aaattccagc tgacgagata aagaaagagt 240
tgaaagctgt tggtctatct caagaggctg tccaggagct attgcaagtc ctttctgtga 300
agtcattgac cgagttagaa gagagacttg ggagagtggg gaagcagttg ctgatctgaa 360
acagtattct cccttgctga aaaaattggt tactctaaat ggttcaattt gatgatagtt 420
gttcgaggtc ttgcttacta cactggcatt gatttgaggg tttgacgaga ggaagctgca 480
gcntctgtgt gtgtcaatac attgnn 506

<210> 36
<211> 48
<212> PRT
<213> Glycine max

<400> 36
Asp Val Gly Phe Lys Val Ser Ser Arg Lys Val Leu Gln Glu Val Leu
1 5 10 15

Asn Cys Tyr Ser Val Pro Glu Asn Leu Phe Gly Lys Val Cys Val Ile
20 25 30

Ile Asp Lys Ile Glu Lys Ile Pro Ala Asp Glu Ile Lys Lys Glu Leu
35 40 45

<210> 37
<211> 577
<212> DNA
<213> Triticum sp.

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<221> unsure
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<222> (564)

<400> 37
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gtgttggata tgtactccgt accacaacac ttgtttactc aagtttgtgt tattgttgac 120
aagctgggga aactgagtan ggaagaaatt gagaaggaat tgatttcaac tgggctgtca 180
tctgaagcag tacagggcat cattgaagtg ctctctctca agtcactgtc caaacttgaa 240
gaggtgctag gctcaggtgt tgaagccgtt gctgacttga agaacctctt ctcgcttgct 300
gagcaatatg gttattctga ttggatctgt ttcgatgcat ctgttgttcg tggccttgca 360
tactacacan gggattgttt ttgaggcttt tgatagggaa gggaaactga nancatttgt 420
ggtgggggggg aggtatgaca ggctacgtca acatttggaa ctgaagatnt ccaccctgtg 480
nctttggatt tggaatcctg tcanagtgga ctccnaaaga aaggtctttn ctacctgcac 540
tcaaataata nattgntcca ttgncaagac ttggggg 577

<210> 38
<211> 46
<212> PRT
<213> Triticum sp.

<220>
<221> UNSURE
<222> (38)

<400> 38
Ile Arg Leu Ser Ser Arg Lys Val Leu Gln Ala Val Leu Asp Met Tyr
1 5 10 15

Ser Val Pro Gln His Leu Phe Thr Gln Val Cys Val Ile Val Asp Lys
20 25 30

Leu Gly Lys Leu Ser Xaa Glu Glu Ile Glu Lys Glu Leu Ile
35 40 45